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Novel therapeutic interventions for pseudoachondroplasia

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ABSTRACT

Pseudoachondroplasia (PSACH), a severe short-limbed dwarfing condition, is associated with life-long joint pain and early onset osteoarthritis. PSACH is caused by mutations in cartilage oligomeric matrix protein (COMP), a pentameric matricellular protein expressed primarily in cartilage and other musculoskeletal tissues. Mutations in COMP diminish calcium binding and as a result perturb protein folding and export to the extracellular matrix. Mutant COMP is retained in the endoplasmic reticulum (ER) of growth plate chondrocytes resulting in massive intracellular COMP retention. COMP trapped in the ER builds an intracellular matrix network that may prevent the normal cellular clearance mechanisms. We have shown that accumulation of intracellular matrix in mutant-COMP (MT-COMP) mice stimulates intense unrelenting ER stress, inflammation and oxidative stress. This cytotoxic stress triggers premature death of growth plate chondrocytes limiting long-bone growth. Here, we review the mutant COMP pathologic mechanisms and anti-inflammatory/antioxidant therapeutic approaches to reduce ER stress. In MT-COMP mice, aspirin and resveratrol both dampen the mutant COMP chondrocyte phenotype by decreasing intracellular accumulation, chondrocyte death and inflammatory marker expression. This reduction in chondrocyte stress translates into an improvement in long-bone growth in the MT-COMP mice. Our efforts now move to translational studies targeted at reducing the clinical consequences of MT-COMP and painful sequelae associated with PSACH.

1. Pseudoachondroplasia – the skeletal dysplasia

The first clinical and radiographic description of pseudoachondroplasia (PSACH) was reported in 1959 [1]. Since then, numerous studies of PSACH provide a comprehensive understanding of the natural history of the disorder [2–9]. PSACH babies are indistinguishable from other newborns during the first year of life because they have a normal birth length and weight. Diminished linear growth and/or a waddling gait are the first signs that alert the health care practitioner and/or parents that there is a growth problem. Radiographic examination leads to a diagnosis by the age of 18–24 months based on characteristic x-ray findings including shortening of all the long bones, small abnormal epiphyses, widened and irregular metaphyses, small, underossified capital femoral epiphyses and platyspondyly [3,7,9–11]. During childhood, limb shortening, brachydactyly, widened joints and joint laxity become obvious and lower limb abnormalities develop, ranging from genu varus to genu valgum or a combination of both [9,11]. Lower extremity abnormalities generally require surgical interventions (osteotomies); the timing of the procedures depends on the extent of joint laxity and the degree of deformity. The average adult height is 3′9″–3′11″ which is equivalent to the height of an average 6 year old (https://ghr.nlm.nih.gov/condition/pseudoachondroplasia). However, stature is variable with some being as tall as 4′10″. Early onset osteoarthritis occurs in young adults and produces significant discomfort. This affects all the major joints necessitating joint replacements usually starting with hip replacements in the second to third decades [4,7,11,12]. A recent natural history study found that pain starts in early childhood and is a significant problem for which there is no systematic or standard pain treatments [9,13]. Chronic pain, the most debilitating feature of PSACH, compromises mobility ultimately limiting physical activity and quality of life [7].

PSACH is an autosomal dominant disorder, that occurs as a (*de novo*) new event in 70–80% of families with the remaining cases being inherited from an affected parent [10,13,14]. Although autosomal recessive inheritance was reported based on recurrence in siblings of unaffected parents, these cases were subsequently shown to result from germline mosaicism. Affected individuals have a 50% risk of passing the mutation to their offspring in each pregnancy and prenatal diagnosis is available using molecular testing. Prenatal ultrasound will not detect PSACH since skeletal abnormalities develop postnatally overtime. Prenatal molecular diagnosis will establish affection status for familal cases.

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2. Mutations in cartilage oligomeric matrix protein (COMP) cause PSACH

PSACH was first described as an rough endoplasmic reticulum (rER) storage disorder in 1972 based on electron micrography studies of iliac crest biopsies [3,15,16]. These studies revealed retention of a lamellar-appearing material in massively dilated ER cisternae of growth plate chondrocytes [17–24]. In 1995, mutations in COMP were shown to cause PSACH and the stored ER material was identified as COMP [10,15]. Since then, more than 200 mutations have been identified with ≅99% found in the the highly conserved calcium-binding repeat domains indicating that this domain is extremely sensitive to genetic alterations (LOVD Mendelian genes https://grenada.lumc.nl/LOVD2/mendelian_genes/variants_statistics.php) [9,25–27]. Approximately 30% of cases result from deletion of one of five sequential aspartic acid residues at position 469–473 and is denoted as the D469del mutation [10].

COMP is a homopentameric protein that has a bouquet appearance on rotary shadowing with the *N*-terminal domain joining the five subunits [28]. Each COMP monomer has four distinct domains: *N*-terminal pentamerization domain, epidermal growth factor (EGF)-like domain (four repeats), a type 3 calcium-binding domain (7 repeats) and a C-terminal lectin-like globular region [29]. Mutations in the calcium-binding domain interfere with the number of bound calciums, which is predicted to disrupt protein folding of each arm thereby having a dominant negative effect on the protein [30]. Chondrocytes from three PSACH patients with different mutations, [D469del, G427E and D511Y], all show similar intracellular retention of COMP in the endoplasmic reticulum [31,32]. Crystallographic studies show that the type 3 calcium binding domain wraps around the calcium metal scaffold in a unique 3D structure and mutations in this domain are predicted to cause a local collapse of the 3D structure [33]. Indeed, functional studies confirm that mutations disrupt calcium binding and protein folding with the mutant arms measuring longer that the wild type arms [28].

3. Function of COMP

COMP was first isolated from cartilage and thought to be cartilage-specific but was later shown to be relatively abundant in other musculoskeletal tissues such as tendon, ligament and smooth muscle [18,29,34–43]. COMP is a secreted glycoprotein found in extracellular matrices (ECM) and is best characterized in the pericellular and territorial matrices surrounding growth plate and articular chondrocytes [21,40]. COMP, the fifth member of the thrombospondin gene family; also designated as, TSP-5, binds to a number of proteins in the ECM with the C-terminal domain a hub for interaction(s) with other ECM proteins [44–46]. For example, COMP binds to matrillins-1, −3 and −4 (MATN) [19,45], interacts with glycosaminoglycan (GAG) side chains of aggrecan within the type 3 repeats and the C-terminal domain [47]. The C-terminal globular domain binds to types I, II, IX, XII and XIV collagens [44,46,48,49] and may enhance mechanical strength of the ECM and serve as anchor plaques/points for protein complexes. COMP has also been shown, *in vitro*, to increase the speed at which type II collagen fibrils assemble by potentially positioning free collagen molecule close to each other [50]. Other work suggests that the interaction of COMP with collagens organizes the collagen ECM network (fibril morphology and density) and that COMP is critical for collagen secretion [51]. Interestingly, this observation is consistent with iliac crest growth plate and ligament PSACH biopsies showing varied collagen fibril structure, diameters [52] and fused fibrils [53]. Moreover, a recent study of COMP null mice showed showing alterations in collagen fibrils in skin and tendon [51] while an earlier study did not [54]. These studies support the idea that collagen assembly in the matrix is affected by the absence of COMP in the matrix either by knocking it out or because of retention within the chondrocytes.

Fibronectin binds to integrins, which are anchored in the cellular membrane, and fibronectin also interacts with COMP at the C-terminal domain [55]. COMP directly facilitates chondrocyte attachment through interaction specifically with α5β1 and α5β3 integrins [56]. The interaction of these integrins with the RGD sequence in the calcium binding repeats are critical for chondrocyte attachment [56]. Granulin epithelin precursor (GEP), a growth factor, interacts with the EGF domain of COMP and this interaction stimulates chondrocyte proliferation [57]. The precise function of the EGF domain in this context is unclear but the domain includes six cysteine residues involved in disulphide bonds which are common in secreted proteins (http://smart.embl.de/smart/do_annotation.pl?DOMAIN=SM00181). Collectively these findings suggests that COMP may be a structural component of cartilage also involved in regulating chondrocyte function.

The specific role of COMP in different tissues is not well defined. Weight bearing equine tendons produce more COMP than non-weight bearing ones suggesting that COMP may play a role in withstanding mechanical stress consistent with the presence of a mechanosensitive

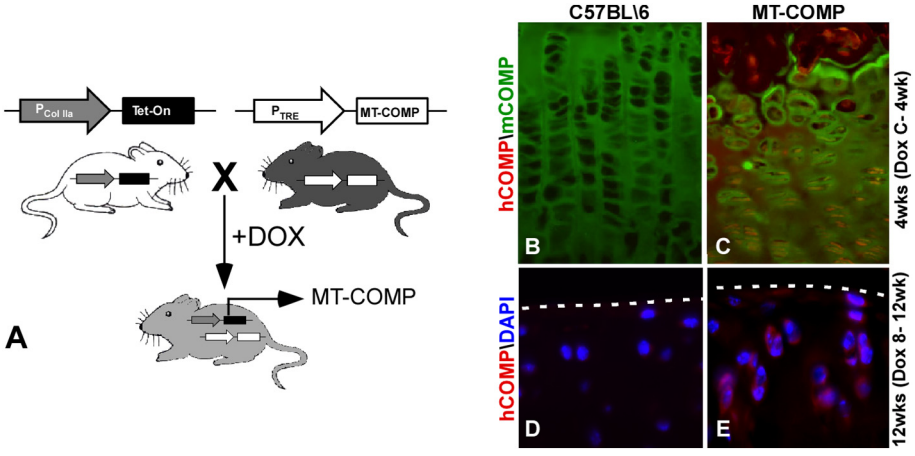

Fig. 1. MT-COMP transgenic mouse. (A) MT-COMP bigenic mouse line was generated using the Tet-On system with the tetracycline responsive element (TRE) driving human D469del-MT-COMP expression. The type II collagen promoter (Col IIa) drives rtTA (Tet-On) expression in chondrocytes. (B) In the presence of doxycycline (DOX), the bigenic MT-COMP mouse expresses mutant human D469del-COMP in growth plate and articular chondrocytes as visualized using human-specific COMP antibody. MT-COMP (hCOMP in red) expression is detected in the murine growth plate chondrocytes at 4 weeks with administration of DOX from conception to 4 weeks (C-4wk) (C), whereas mouse COMP (mCOMP in green) is primarily extracellular (B and C). Similarly, MT-COMP is found in articular cartilage chondrocytes of 12 week mice administered DOX from 8 to 12 weeks (E) but not in controls (D). The articular cartilage border is marked by a dashed line. DAPI staining of nuclei are shown in blue (D and E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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