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Characterization of Structural Changes in the Subchondral Bone and Fibrocartilage from a Mouse Model of Temporomandibular Joint Osteoarthritis

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Characterization of Structural Changes in the Subchondral Bone and Fibrocartilage From a Mouse Model of Temporomandibular Joint Osteoarthritis

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Characterization of Structural Changes in the Subchondral Bone and Fibrocartilage From
a Mouse Model of Temporomandibular Joint Osteoarthritis

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2008
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Abstract

Introduction: Temporomandibular disorders are a collective term embracing a number of clinical problems that involve the masticatory musculature, the temporomandibular joint (TMJ) and associated structures or both. One class of temporomandibular disorders is degenerative diseases, specifically osteoarthritis. Previously, many have characterized subchondral bone and cartilage of joints such as the knee; however, few have investigated the temporomandibular joint. Consequently we used the previously defined biglycan-fibromodulin double-deficient mice to evaluate the changes of osteoarthritis in the condylar cartilage and bone in 3 and 9 month old mice compared to wild-type controls (WT).

Materials and Methods: A total of 71 mice were divided into 4 groups at 2 time points, 3 months and 9 months. The two groups consisted of wild-type and double-deficient mice and further sub-divided into male and female. The subchondral bone of the mandibular condyle was evaluated by micro-CT analysis and gene expression was evaluated by real time PCR analysis.

Results: The Micro-CT data comparing 3 month WT versus biglycan/fibromodulin double deficient mice showed that there was no statistical difference between trabecular thickness, trabecular number, trabecular spacing, total volume, bone volume, and bone surface. In the 9 month data, comparison showed an increase in bone volume, total volume, bone surface, trabecular spacing, and trabecular number and a decrease in trabecular thickness in Double Deficient mice. In the 3 month double deficient mice, there was a decrease in Osteocalcin and PTHrP and at 9 months there was an increase in Col X and Aggrecan. No changes were seen in the wild-type mice. Three month old mice
had an increase in condylar cartilage collagen fibril diameter thickness compared to the wild-type controls.

**Conclusion:** We see condylar cartilage changes that precede subchondral bone changes in the biglycan/fibromodulin double deficient mice.
Introduction

Temporomandibular disorders (TMD) are a collective term embracing a number of clinical problems that involve the masticatory musculature, the TMJ and associated structures or both. The most frequent presenting symptom is pain, usually localized in the muscles of mastication, the pre-auricular area, and/or the joint. The National Institute of Dental and Craniofacial Research of the National Institutes of Health reported that 10.8 million people in the United States suffer from TMJ problems at any given time. Signs and symptoms of TMD observed in childhood increase in frequency and severity beginning in the second or third decade of life. TMJ disorders affect men and women; however, 80% of those who seek treatment for the disorders are women. It is estimated that 17,800,000 work days are lost each year for every 100,000,000 full-time working adults in the United States due to disabling TMD. One type of disorders are degenerative diseases of the TMJ(TMJ-OA). Approximately 50 % of all patients who have TMJ disorders have TMJ-OA. TMJ-OA is characterized by an imbalance between chondrocyte production and degradation in the temporomandibular joint, resulting in a progressive degradation of extra-cellular matrix components of the articular cartilage and/or subchondral bone. In this chapter we will review the etiology behind TMJ-OA, imaging for TMJ-OA and efforts under way into reconstructing a new TMJ.
1. TMJ Overview Anatomy

Anatomy

Mandibular condylar cartilage

The temporomandibular joint (TMJ) is considered to be a ginglymoarthroidal joint: a joint that is able to have both rotational and translational movements. The TMJ is formed by the mandibular condyle fitting into the mandibular fossa of the temporal bone. Separating these two bones from direct contact is the articular disc. The articular portion of the disc is comprised of dense fibrous connective tissue, devoid of any nerves and vessels; conversely, the posterior attachment of the disc is richly vascularized and innervated. The disc is attached to the condyle both medially and laterally by collateral ligaments. Rotational movement occurs between the condyle and the inferior surface of the disc during early opening (the inferior joint space) and translation takes place in the space between the superior surface of the disc and the fossa (the superior joint space) during later opening. Synovial fluid within the joint facilitates movement within the joint; it also functions as a medium for transportation of nutrients to and waste products from their articular surfaces.

The mandibular condylar cartilage is composed of four distinct layers or zones. The most superficial layer is called the articular zone or superficial zone. It is found adjacent to the joint cavity and forms the outermost functional surface. This zone is responsible for dissipating shearing and frictional loads generated by jaw functions. One way it is able to accomplish this is by the expression of a protein named superficial zone protein (SZP). In the TMJ, SZP localizes to the superficial layer of the mandibular condylar
cartilage and the TMJ disk [1]. Superficial zone protein (SZP) is a large proteoglycan that is synthesized and secreted into synovial fluid and is known to function as a boundary lubricant in articular joints by reducing the coefficient of friction of the mandibular condylar cartilage surface and the strain energy of the synovial fluid [2].

The second zone is the polymorphic or proliferative zone; this zone is mainly cellular with undifferentiated mesenchymal tissue. The cartilage cells in this layer are large and are enclosed in lacunae. There is no organization in formation or arrangement of cartilage cells in this layer. This tissue is responsible for the proliferation of articular cartilage in response to the functional demands placed on the articular surfaces during loading and unloading. This zone is characterized by the expression of sox 9 and the absence of the expression of collagen type II [3].

The third zone is the flattened or chondroblastic zone. The cartilage cells in this region are highly mature. The cartilage cells have not yet lost their ability to proliferate [4]. In this zone the collagen fibrils are arranged in bundles in a crossing pattern. The fibrocartilage appears in a random orientation, providing a three-dimensional network that offers resistance against compressive and lateral forces. Cells in this layer are characterized by the expression of sox-9, collagen type I, II and Indian hedgehog [3].

The fourth zone and deepest zone is the hypertrophic zone. In this zone, the chondrocytes become hypertrophic and die. Cartilage breakdown occurs and cartilaginous spicules undergo calcification with hydroxyapatite crystals. The surface of the extracellular matrix scaffolding provides an active site for remodeling activity as endosteal bone growth proceeds. Deeper into the zone bone and marrow spaces are present. The bony trabeculae are arranged randomly and not perpendicular to the
articulating surface. Cells in this zone are characterized by the expression of Indian hedgehog, osteopontin, and collagen type X [3].

**TMJ Disc**

The TMJ disc is an articular disc composed of dense fibrous connective tissue, which is primarily deficient of any blood vessels or nerve fibers. The disc lies in between the condyle of the mandible and the mandibular fossa of the temporal bone. The disc divides the joint cavity into two distinct components: the upper compartment and the lower compartment. The upper or superior cavity is delineated by the mandibular fossa and the superior surface of the disc. The lower or inferior cavity is bordered by the mandibular condyle and inferior surface of the disc. Each compartment is filled with a plasma-like synovial fluid secreted by cells of the synovial lining. Synovial fluid serves as a multi-purpose substance. Since the articular disc and the articular surfaces of the joint are devoid of vasculature, the synovial fluid acts as a vehicle for providing metabolic requirements for these tissues. Also, the synovial fluid aids in minimizing friction by serving as a lubricant in between all these articular surfaces [5]. The morphology of the disc matches the shape of the condylar head and the mandibular fossa. In the frontal view, the disc is concave inferiorly designed to fit over the condylar head [6]. The articular disc is attached firmly to the medial and lateral poles of the condyle in order to prevent the disc from excessively moving during condylar movements. In the sagittal plane it can be divided into three regions according to thickness. The central area is the thinnest, and the anterior and posterior regions are much thicker in comparison. From the anterior view, the disc is thicker medially than laterally. The articular disc is attached
posteriorly to a region of loose connective tissue, known as the retrodiscal tissue, which is highly vascularized and innervated [6]. Cells of TMJ disc are characterized by the expression of versican [7] and little expression of collagen type II [8]. Interestingly, the TMJ disc does not form in mice deficient for Indian hedgehog [3].

TMJ is innervated by the same nerve that provides motor and sensory innervation to the muscles that control it, the trigeminal nerve. Most of the innervation is provided by the auriculotemporal nerve; additional innervation is provided by the deep temporal and masseteric nerves. The TMJ is richly supplied by the superficial temporal artery from the posterior, the middle meningeal artery from the anterior, and the internal maxillary artery from the inferior. The condyle receives its vascular supply through its marrow spaces by way of the inferior alveolar artery.

**Uniqueness of the TMJ**

The TMJ is different in composition and developmental than other joints in the body. Therefore, it is not surprising that there are certain diseases that affect every joint in the body except for the TMJ. In other synovial joints in the body, the articular surfaces are covered by hyaline cartilage. The TMJ is different because it is composed of fibrocartilage (see [9] for a review). One of the unique property of fibrocartilage is that it contains both Type I and Type II collagen compared to articular hyaline cartilage, which only contains Type II collagen [10]. Fibrocartilage is better able to withstand sheer forces than hyaline cartilage, which is essential in the large amount of occlusal load that
is placed on the temporomandibular joint [11]. Other advantages of fibrocartilage in the TMJ over hyaline cartilage are that the fibers are tightly packed and are able to withstand the forces of movement, it is less susceptible than hyaline cartilage to the effects of aging and is less likely to break down over time and it has a better ability to repair than hyaline cartilage [12].

Another difference between the TMJ and other joints is that the cartilage of the mandibular condyle is a secondary cartilage compared to the articular cartilage found in other joints, which are primary cartilage [13]. More specifically, secondary cartilage develops in association with specific bones formed by intra-membranous ossification after the bones are already formed. This is different from cartilage associated with endochondral ossification, where the cartilage precedes the bone formation and is referred to as a primary cartilage. Primary cartilage growth begins in the cartilage cells within the central layer of an epiphyseal plate. In this developmental stage, the cells undergo mitosis. The two daughter cells will contain the total amount of genetic information from the original cell. In the next phase of epiphyseal growth is the enlargement of the two daughter cells to the size of the original. Each cell produces and secretes extra-cellular matrix which causes the cells to drift away from each other and to enter various pathways. The cells may either become a new progenitor cell or be replaced by bone. One of the key elements of primary cartilage growth is that growth occurs in the middle part of an epiphyseal plate of a long bone; when new growth occurs within existing tissue this is termed as interstitial growth [14] [15].

Secondary condylar cartilage growth beings with undifferentiated cells comprising mesenchymal tissue, covering the pre-natal or post-natal condyle. In the developmental
stages, the mesenchymal cells divide themselves becoming even smaller cells, but eventually attaining full size. These mesenchymal cells then migrate into the interior of the condyle, into the cartilage where differentiation occurs and the cells become immature cartilage cells [15]. The growth in the cartilage has occurred through differentiation of mesenchymal tissue rather than mitosis of cartilage progenitor cells. This type of growth where growth occurs from the exterior is known as appositional growth [14].

Two diseases that affect every load-bearing joint during growth in the body but the TMJ are: Hunter-Thompson chondrodysplasia and FGF-3 achondroplasia. Cartilage-derived morphogenetic protein-1 (CDMP1), a member of the bone morphogenetic protein superfamily, is located and expressed in the synovial-lined load-bearing joints during embryological development. This protein has high expression levels in the distal parts of limbs, suggesting an important role of CDMP-1 in the development of the appendicular skeleton and joint morphogenesis [16]. The effects of this protein are profoundly delineated in Hunter-Thompson chondrodysplasia. Individuals with this syndrome have dysmorphic joints and are usually short in stature. Hunter-Thompson chondrodysplasia results from a 22-base pair frameshift mutation of the cdmpl gene, resulting in a complete loss of function of the growth factor. Interestingly, this gene affects all load bearing joints in the body with the exception of the temporomandibular joint [16].

Another growth plate disease that affects many joints of the body is achondroplasia and results in dwarfism. Achondroplasia results from a defect in cell signaling, with a subsequent reduction of chondrocytes in the growth plate. Patients with this disorder have a point mutation in arginine or glycine in the gene that codes for FGF
receptor 3. Under normal conditions, FGF-3 receptor activation inhibits cartilage proliferation; however, the mutation in this gene causes a gain of function of the FGF receptor causing the receptor to be in constant activation. Phenotypically the genetic disorder is expressed as shortened proximal extremities and an enlarged head with bulging forehead and depression of the root of the nose [17]. Interestingly once again, although most other joints in the body are affected by this point mutation, condylar cartilage does not seem to be affected [18].

As outlined above, even though not well comprehended there are differences between the TMJ and other joints. Therefore broad assumptions from articular hyaline cartilage to explain or to find appropriate treatments for TMJ disorders are not merited.

2. Pathogenesis of TMJ-OA

Osteoarthritis occurs from the lack of equilibrium of anabolic and catabolic processes of chondrocyte initiation, proliferation and differentiation. It is characterized by degradation of the components of the extra-cellular matrix and its associated secondary inflammatory mediators. The initial signs of osteoarthritis may be sub-clinical; consequently, onset of symptoms may not present until later stages of development of the disease. The classification of the pathogenesis of osteoarthritis has been divided into 4 stages.

The first stage is classified as initial and repair stage of OA (osteoarthritis). If a primary insult alters the chondrocyte mediated balance between synthesis and degradation of the extra-cellular matrix, then cartilage degradation occurs. At first, the
cartilage will try to repair itself by internal mechanisms, by increased production of extra-
cellular matrix components and DNA resulting in proliferation, mitoses, and increased
metabolic activity of the chondrocytes observed histologically. The changes seen in the
cartilage in the initial and repair stage may remain asymptomatic for years [19].

The second stage is known as the early stage of osteoarthritis. The imbalance between
synthesis and degradation of extra-cellular matrix components first appears. An increase
in degradation occurs due to an increase in synthesis and activity of proteases which
results in a degradation and loss of articular cartilage. Irregularities in the cartilage
surface appear with areas of focal swelling and necrosis [20]. The collagen network
shows signs of disorganization, in the TMJ and the fibrocartilgae. In the electron
microscope, the collagen fibrils of the articular surface are disoriented and more widely
separated than normal.

The third stage in this pathological process is known as the intermediate stage. The
synthesis of extra-cellular components fails, and the synthesis and the activity of
proteases remains increased which produces progressive degradation and loss of articular
cartilage. The collagen network shows signs of advanced disorganization. Electron
microscopy shows the collagen fibrils to be loose and disoriented, with an increase in
elastic fibers [21]. In this stage of osteoarthritis, the TMJ appears as a thin, fibrillated
tissue. The fibrillation may appear to be focal or extensive. The loss of smoothness of the
joint, may lead to subsequent disc displacement [21]. The synovial membrane may
appear hypervascularized, hypertrophic, or fibrotic. In this stage, the patient may begin to
clinically feel pain in the joint, experience limited jaw movement, or noises may be heard
due to disk displacement.
In the late stages of osteoarthritis, there is a marked reduction in extra-cellular matrix components—such as water, proteoglycans, and collagen. The synthesis of proteases remains high, and the synthesis of protease inhibitors remains low. Histologically, the TMJ appears to have extensive fibrillation and denudation of the subchondral bone. In electron microscopy, the collagen network is extensively disorganized and disintegrated. The patient may feel extreme pain in the TMJ, and increased limitation of movement of the jaw [22].

3. Biglycan/Fibromodulin Double KnockOut TMJ-OA Mouse Model

One TMJ-OA mouse model involves deficiencies in the production of two extracellular matrix proteoglycans, biglycan (BGN) and fibromodulin (FMOD). At 6 months of age, osteoarthritic changes first begin to develop in the TMJ and progressively get worse and by 18 months, the double deficient mice have almost complete destruction of the TMJ [23]. Biglycan is a small proteoglycan abundant in cartilage, bone, and other connective tissues whose function is not yet understood. Fibromodulin is a collagen binding protein that is homologous to biglycan structurally, insinuating that both proteoglycans arose from the same ancestral gene. Fibromodulin was isolated from cartilage and is present in many types of connective tissue including, cartilage, tendon, skin, sclera, and the cornea. These proteoglycans have several properties that are important in the resiliency of articular cartilage, and aid in providing water for surface lubrication. These proteoglycans are associated with collagen and may aid in directing the formation and spatial orientation of the collagen fibrils [24]. Although the exact
mechanism underlying TMJ-OA is unknown, the absence of biglycan/fibromodulin may cause changes in the extracellular collagen network making the joint less susceptible to withstand mechanical loading and subsequently more prone to osteoarthritic lesions [23]. In support of this, in the knee of double knockout biglcyan/fibromodulin mice, increased mechanical loading by forced treadmill running caused an increased severity of OA [25]. In another explanation, changes in the TMJ fibrocartilage arise from the ability of biglycan/fibromodulin to modulate members of the transforming growth factor (TGF)-β superfamily. The binding of biglycan and fibromodulin to members of the TGF-β family may regulate their activity by sequestering them into the ECM, thereby preventing their binding with the cellular receptors. Evidence has been shown by examination of another small leucine rich proteoglycan-deficient mouse. Bi et al found that the bone marrow stromal cells from biglycan/decorin double deficient mice exhibited an increase in TGF-β activity [26].

4. Changes in Micro-architecture of Cartilage and Bone in Mouse Models

In late stages of osteoarthritis, changes in the joint affected by osteoarthritis can be visible on radiographs. The sclerosis of the subchondral bone is one of the hallmarks of the radiologic diagnoses of osteoarthritis. There has been a lot of controversy over the destruction of cartilage and bone and which changes occur first. Some research has shown that thickening of the subchondral bone occurs before alterations in the cartilage [27, 28]. Yet others argue that thickening of the bone does not have to precede cartilage fibrillations [29].

Micro-CT
Among animal models and human bone diagnosed with osteoarthritis, there have been many studies that have shown an increase in trabecular bone volume in affected joints. Previously the results from 2D slices of histological cross-sections remain controversial; however, of recent the subchondral bone architecture can be evaluated precisely from 3D micro-computed tomography. To date most studies have examined the structural changes of the bone in the knee and hip joints, and very few have looked at the temporomandibular joint. Fazzalari and Parkinson have found that there is an increase in trabecular bone volume in fovea from osteoarthritic hip [30]. Ding et al concluded that cancellous bone in osteoarthritic humans was markedly thicker and plate-like but lower in mechanical properties than normal bone. Ding et al looked at changes in the three-dimensional microstructure of proximal tibiae induced with osteoarthritis using Micro-CT. They found that although the bone mineral density increases in the axial and peripheral skeleton with the pathogenesis of osteoarthritis, there may be a low mineralization pattern in the late stages of OA. Earlier stages of OA show a decrease in bone volume and a resultant increase in bone volume as time increases. Bobinac et al. compared tibial plateaus of patients with severe osteoarthritis and those without the disease, controls. This study showed there was a significantly higher bone volume in the subchondral bone of the osteoarthritis group versus the control group. Trabecular parameters from the osteoarthritic group indicated thicker and sparser trabeculae [31]. Again many studies have been performed in the hip and knee joint, yet relatively little data exist for the TMJ.
Transmission Electron Microscopy

Collagen fibrils have a dual role of retaining the proteoglycans in the extra-cellular matrix and contributing to the mechanical function of articular cartilage as a load-bearing tissue. Consequently a disorganization of the collagen fibril matrix can lead to altering load-bearing tissues and specifically the mechanical function of the articular cartilage. Although many studies have examined the changes in the knee joints of mice induced with osteoarthritis, very few have looked at the temporomandibular joint of diseased mice. In a study by Xu et al, they remarked on an increase in collagen fibril diameter in articular cartilage of knee joints of 3 month old heterozygous cho/+ mice as compared to controls. The changes in the collagen fibril spacing increased even more with aging[32]. Alternatively, Ameye et al looked at the biglycan/fibromodulin knockout mouse model of osteoarthritis. They noticed that in tendons of mice deficient in these proteoglycans, collagen fibrils are structurally and mechanically modified causing unstable joints. The mice develop gait impairment, ectopic tendon ossification, and severe premature osteoarthritis. The electron microscopy analysis revealed an increased number of collagen fibrils and collagen fibrils with very small diameters [25]

Gene Expression

Several studies of the knee joint have quantified gene expression through Real Time-PCR of the condylar cartilage of osteoarthritic animal models. In a sample of mature vs aged rabbits, with osteoarthritis induced with anterior cruciate ligament resection, it was shown that Fas, Caspase 8, and MMP-13 were elevated in the rabbits with induced OA [33]. In yet another study on dogs, the authors quantified genes that may be expressed in early versus late stage osteoarthritis. Early phase OA markers
include \textit{col II}, \textit{col I}, and \textit{YKL40} and late stage markers include \textit{tenascinC} and \textit{MMP-13} [34]. In the TMJ, Meng et al show that in induced OA by means of alterations in the TMJ disc, rats show an increased expression of \textit{EGLN3}, \textit{MMP3}, \textit{AQP3}, \textit{NOV}, \textit{DKK3} [35].

In the murine model, gene expression SRY box-containing gene (\textit{Sox-9}) is increased, collagen type II (\textit{Col II}) is increased [36], osteoprotegerin (\textit{Opg}) is increased [37], vascular endothelial growth factor (\textit{Vegf}) is increased [38], and collagen type X (\textit{Col X}) is decreased [39].

\textit{Rationale}

The differences between the TMJ and other joints is not well comprehended, resulting in broad assumptions from articular hyaline cartilage to explain or to find appropriate treatments for TMJ disorders. Changes in the microarchitecture of the subchondral bone and cartilage have been described in mouse models of knee osteoarthritis but not in the TMJ. Wadhwa et al [23] showed mice developing osteoarthritis by 6 months; however, the subchondral bone and fibrocartilage changes were not previously characterized. Based on the absence of a bone phenotype in our mice, the purpose of this study is to examine the subchondral bone and fibrocartilage changes of the condylar head of 3 and 9 month old biglycan/fibromodulin double deficient mice. Since the temporomandibular joint has a limited capacity to regenerate, it would be beneficial to detect TMJ-Osteoarthritis before irreversible joint damage.
**Hypothesis**

We have recently described an osteoarthritis TMJ mouse model that develops OA lesions in the TMJ at 6 months of age in mice. In this study we want to examine if the absence of biglycan and fibromodulin causes an increase in the collagen fibril diameters in the condylar cartilage and/or an increase in the bone volume and trabecular thickness in the subchondral bone before and during active OA.

The specific hypotheses to be tested in this project are:

1. We expect to see an increase in collagen fibril diameters in the condylar cartilage which will precede an increase in the subchondral bone, specifically bone volume and trabecular thickness in the male and female Bgn/Fmod 9month Double Deficient mice.

2. We expect to see an increase in bone volume and trabecular thickness in 9 month old Double Deficient mice compared to Wild Type mice.

3. We expect to see altered gene expression levels in the 9month old Double Deficient mice vs the 9month WT mice.
SPECIFIC AIMS/ OBJECTIVES/ PREDICTIONS

Aim 1. **Determine the difference of collagen fibril diameter of the mandibular condylar cartilage from 3 and 9 month old WT and Double Deficient mice.**

We hypothesize that the increase in collagen fibril diameter will precede overt OA changes in the condylar cartilage from Double Deficient mice. In the Double Deficient mice, OA lesions will first become apparent when the mice are 6 months of age. In this aim we will examine 3 month and 9 month old WT and Double Deficient Mice mice. There will be 2 groups, one control, the wild type and the second experimental, the biglycan/fibromodulin double knockout mice. At 3 months, there is an n=6 in both the wild-type and double deficient groups. At 9 months, there is an n=6 and n=8 in both the wild-type and double deficient groups respectively. The condyles of the mice will be dissected and placed in containers filled with a fixative solution-2.5% glutaraldehyde and 2% para-formaldehyde. The transmission electron microscopy will be performed by Dr. Arthur Hand at the University of Connecticut Health Center. The mandibular condylar cartilage of wild-type and double deficient mice will be evaluated for a quantitative analysis of fibril diameters consisting of mean, median, range and a distribution profile. We expect to see an increase in the collagen fibril diameters and spacing in the mandibular condylar cartilage from 3 month old double deficient mice compared to wild-type controls, which will progress in an age dependent manner.

Aim 2. **Evaluation of the subchondral bone of the mandibular condyle from male and female 3 month and 9 month old WT and Double Deficient mice**
It has been hypothesized that osteoarthritis causes an increase in the underlying bone. Consequently, in this aim we will examine the subchondral bone of the mandibular condyle of male and female wild-type and double deficient mice at 3 and 9 months by Micro Computed Tomography (micro-CT). There will be 2 groups, one control, the wild type and the second experimental, the biglycan/fibromodulin double knockout mice. At 3 months, there is an n=6 in both the wild-type and double deficient groups. At 9 months, there is an n=6 and n=8 in both the wild-type and double deficient groups respectively. We hypothesize that there will be an increase in bone volume and trabecular thickness in the subchondral bone only after overt TMJ-OA lesions. Therefore we do not expect to see a difference in trabecular thickness and bone volume in the subchondral bone from 3 month old double deficient mice but do expect to see a difference in 9 month old mice. Multi-scanning of the entire mandibular condyle will be performed by 3D micro-CT and reconstituted into 3D images. Volume cubes of a fixed quantity will be taken in a sagittal plane from trabecular bone just below the cartilage layer in the superior part and at the posterior end of the mandibular condyle. Measurements of the following will be made using micro-CT: bone surface (BS), the total surface square measure of the trabeculae in the fixed volume cube, bone volume (BV), the total volume of the trabeculae in the fixed volume cube, trabecular number (TbN), the total number of trabeculae in the fixed volume cube, trabecular thickness (TbTh), the mean trabecular thickness in the fixed volume cube; and trabecular separation. Dr. Doug Adams at the University Connecticut Health Center will perform the micro-CT analysis. We expect to see an increase in bone volume and trabecular thickness in the micro-architecture of the subchondral bone only after mandibular condyle active osteoarthritis.
Aim 3: Evaluation of the Gene Expression of 9month BGN/Fmod Double Deficient mice vs 9month old WT mice.

We expect to see altered levels in gene expression specifically in the 9 month old wild-type mice versus the double deficient mice. We do not expect to see any changes in the 3 month old double deficient or wild-type control mice because we do not believe that they have developed temporomandibular joint osteoarthritis as shown in previous models.

Real Time PCR will be performed on samples (n=45) from both time points, 3 and 9 months. At 3 months there is an n=10 in both wild-type and double deficient groups and at 9 months there is an n=13 and n=12 respectively in wild-type and double deficient groups. mRNA will be extracted from both the condylar head which includes cartilage and subchondral bone. The following genes will be evaluated at both time periods: 

*Collagen 1, Collagen 2, Collagen X, Aggrecan, Osteopontin (OPN), Osteocalcin (OC), Parathyroid hormone related protein (PTHrP), Indian Hedgehog (Ihh), VEGF, Sox-9, Runx-2.*
Chapter 2

Characterization of Microarchitectural Changes in the Condylar Cartilage and in the Subchondral Bone In a Temporomandibular Joint Osteoarthritis Mouse Model

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Running Title- Osteoarthritis TMJ Mouse Model

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Conflict of Interest Page

All authors have no conflict of interest
Abstract-

Introduction: Approximately 1% of the US population has osteoarthritis of the temporomandibular joint. Little is known about the natural progression of the disease process. Therefore, the goal of this study is to examine the early microarchitectural and molecular changes in the condylar cartilage and subchondral bone in the biglycan/fibromodulin (Bgn/Fmod) double deficient mouse, which develop osteoarthritis of the temporomandibular joint at 6 months.

Materials and Methods: The temporomandibular joints from 3 and 9 month old wildtype (n=35) and Bgn/Fmod double deficient (n=36) mice were evaluated by micro-CT analysis, transmission electron microscopy and real time PCR analysis.

Results: At 3 months, Bgn/Fmod double deficient mice showed an increase in the collagen fibril diameters of the condylar cartilage and a decrease in the expression of Osteocalcin and PTHrP in the mandibular condylar head compared to WT controls. At 9 months, in the subchondral bone, there was an increase in bone volume and in total volume, while in the mandibular condylar head there was an increase in the expression of Col X and Aggrecan in the Bgn/Fmod double deficient mice compared to the WT controls.

Conclusion: Changes in the microarchitecture of the condylar cartilage preceded changes in the subchondral bone during osteoarthritis in the temporomandibular joint in the Bgn/Fmod double deficient mice.
**Key words**- temporomandibular joint; mouse model; osteoarthritis; biglycan;

fibromoulin; subchondral bone: cartilage
Introduction

The National Institute of Dental and Craniofacial Research of the National Institutes of Health reported that 10.8 million people in the United States suffer from TMJ problems at any given time and TMJ disease is the second most common musculoskeletal disease. One type of TMJ disease is osteoarthritis (TMJ-OA) (Okenson, 1998). Currently, there are no biomarkers for detection of TMJ-OA and no treatment modalities besides palliative relief of the symptoms. Imaging techniques can be used to diagnose TMJ-OA, but only after the temporomandibular joint has been subjected to irreversible damage. The temporomandibular joint has a limited capacity to regenerate, thus it would be beneficial to diagnose this disease process at an early stage. Biopsy specimens of the human pre-osteoarthritic TMJ are impossible to obtain, consequently animal models are used to study the entire disease process (Wadhwa et al., 2005).

Several animal models have been developed to study the effects of TMJ osteoarthritis. These TMJ-OA models can be divided into two categories: naturally-occurring (Silbermann, 1979), (Rintala, 1997), (Xu et al., 2003) and trauma induced (Tominaga, 2002),(Fujisawa, 2003),(Harper, 2001). Naturally occurring TMJ-OA models can either be wild-type or transgenic mice that develop osteoarthritis without any intervention. They encompass the entire disease process, allowing one the ability to examine changes that precedes overt osteoarthritis. One such naturally occurring TMJ-OA mouse model is mice deficient in both biglycan and fibromodulin (two small leucine-rich proteins essential in extracellular matrix organization, tissue repair and metastasis) (Ameye et al., 2002). In this model, mice develop TMJ-OA at 6 months and the disease
process progresses with age (Wadhwa et al., 2005). The purpose of our study is to examine if there are structural and/or molecular changes in the mandibular condylar head, which precede overt TMJ-OA. Greater understanding of the molecular and structural changes, which occur in early osteoarthritic TMJ samples, is critical in developing treatment modalities, which prevent or reverse the disease process.

**Materials and Methods**

**Mice**

All experiments were performed under an institutionally approved protocol for the use of animals in research (University of Connecticut Health Center 2005-195). B6/129 wild-type mice were obtained from Jackson Laboratory (Bar Harbor, Maine) and the double deficient biglycan/fibromodulin mice in the B6/129 background were obtained from Dr. Marian Young (National Institute of Dental and Craniofacial Research, Bethesda, MD) (Wadhwa et al., 2005), (Ameye et al., 2002). A total of 71 mice were used in this experiment and the mice were euthanized at 3 or 9 months of age.

**Micro-CT**

The three-dimensional morphometric analysis of the subchondral bone of the mandibular condylar head was evaluated using microcomputed tomography (micro-CT) (µCT 20, Scanco Medical AG, Bassersdorf, Switzerland) by the micro-CT facility at University of Connecticut Health Center headed by Dr. Doug Adams. Mandibles from 3 month WT (n=6), 3 month Double Deficient (n= 5), 9 month old WT (n=6), and 9 month Double Deficient (n=7) mice were dissected and bisected at the level of the symphysis and stored in 70% ethanol. The analysis of the mandibular condylar head included the
bone surface, bone volume, total volume, trabecular number, trabecular thickness, and trabecular spacing.

**Transmission Electron Microscopy**

Mandibles were dissected from 3 month old WT (n=7), 3 month old Double Deficient (n=6), 9 month old WT (n=6), and 9 month old Double Deficient (n=8) mice. The mandibles were cut into two halves and placed into 2.5% glutaraldehyde/2.0% paraformaldehyde, buffered to a pH 7.3 with 0.1M sodium cacodylate. The hemimandibles were placed in the fixative within minutes of sacrifice. The fixation proceeded for 24 hours at 4°C. The specimens were then removed from the fixative and placed in 4% EDTA at 4°C with constant stirring for 12 days (Warshawsky, 1967). The condyles were removed from the hemimandibles and further demineralized for an additional 4 days, then rinsed in several changes of cold (4°C) 0.1M cacodylate buffer. The rinsed segments were postfixed with 1% osmium tetroxide in cacodylate buffer at room temperature for 2 hours, dehydrated in a graded ethanol series and embedded in Polybed resin (Polysciences). The condyles were then sectioned sagittally at 1μm and stained with methylene blue/Azure II for light microscopy. Thin sections, 70-90 nm, were cut with a diamond knife, collected on uncoated 200 mesh copper-rhodium grids, and stained sequentially with 1% phosphotungstic acid, 6% uranyl acetate in 50% methanol, and Sato’s lead citrate (Sato et al., 1967). These sections were examined and photographed in a Philips CM10 transmission electron microscope at 60 kV. A total of 27 mandibular condylar heads were examined; ten micrographs of longitudinally oriented collagen fibrils from the middle third of the condylar cartilage were taken at a magnification of 52,000X. The negatives were scanned at a resolution of 1200 pixels per inch in an Epson
Perfection V750 Pro scanner. The images were imported into Adobe Photoshop CS2 (version 9.0.2) and levels and contrast were adjusted. Ten collagen fibrils per image for a total of one hundred collagen fibrils per condylar head were measured using Photoshop. A grid was constructed in Photoshop and ten collagen fibrils were chosen at random and their diameters measured using the measure tool. The examiner was blinded to the samples being measured.

**RNA Extraction and PCR Amplification**

The mandibular condyle was carefully isolated with all the soft tissues removed using a dissecting microscope. Total RNA was obtained from the condylar head, which contains both condylar cartilage and subchondral bone and extracted with TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA) following the manufacturer’s protocol. Total RNA obtained from the left and right TMJ of one mouse was pooled together. Total RNA was converted to cDNA by ABI High Capacity cDNA Archive Kit (Applied Biosystems, Foster city, CA) following the manufacturer’s protocol. Real-Time PCR was performed for expression of different genes in separate wells (singleplex assay) of 96-well plates in a reaction volume of 20 µl. Gapdh was used as an endogenous control. Three replicates of each sample were amplified using Assays-on-Demand Gene Expression for the particular gene of interest, using predesigned unlabeled gene-specific PCR primers and TaqMan MGB FAM dye-labeled probe. The PCR reaction mixture (including 2X TaqMan Universal PCR Master Mix, 20X Assays-on-Demand Gene Expression Assay Mix, 50 ng of cDNA) was run in Applied Biosystems ABI Prism 7300 Sequence Detection System instrument utilizing universal thermal cycling parameters.
For the genes for which the efficiencies of target and endogenous control amplification were approximately equal, relative expression in a test sample compared to a reference calibrator sample (ΔΔCt Method) was used for data analysis. For the genes that were not amplified with the same efficiency as the endogenous control, the Relative Standard Curve method in which target quantity was determined from the standard curve and divided by the target quantity of the calibrator was used. Gene expression was performed for collagen I (Col I), parathyroid hormone related protein (Pthrp), SRY-box containing gene 9 (Sox9), collagen type II (Col II), Indian hedgehog (Ihh), collagen type X (Col X), vascular endothelial growth factor (Vegf), osteopontin (Opn), Aggrecan, and Runx-2.

**Statistical Analysis.**

Statistical significance of differences among means was determined by analysis of variance with post-hoc comparison of more than two means by the Bonferroni method or the Mann-Whitney rank sum test for nonparametric populations using SigmaStat (Jandel Scientific, San Rafael, CA).

**Results**

TMJ sections from three and nine month old WT and Bgn/Fmod double deficient mice were stained with Safranin O. Safranin O is a cationic dye that binds to the negatively charged glycosaminoglycans. At 3 months, there were little apparent differences between the WT and Bgn/Fmod double deficient TMJs (Fig. 1). In contrast, at 9 months of age there were obvious differences due to the accelerated degeneration of the articular cartilages in the bgn/fmod double deficient TMJ (Wadhwa et al., 2005). At this age in the Bgn/Fmod double deficient, small vertical clefts were visible and
chondrocytes started to lose their regular columnar organization and form clusters, while in the WT there was no evidence of either of these two characteristics.

**Changes in Micro-architecture of the subchondral bone during TMJ-OA**

In order to examine the TMJ-OA induced temporal changes in the micro architecture of the mandibular condylar subchondral bone micro-CT analysis was performed. At three months there were no significant differences in any of the micro-CT parameters examined between WT and double deficient mice. However, at 9 months we found a statistically significant decrease in trabecular thickness and a statistically significant increase in trabecular spacing, trabecular number, total volume, bone volume, and bone surface in the $Bgn/Fmod$ double deficient mice versus the wild type controls (Fig. 2).

**Transmission Electron Microscopy**

In order to examine the TMJ-OA induced changes in collagen fibril diameter and organization of the mandibular condylar cartilage, transmission electron microscopy analysis was performed. Measurements of collagen fibrils in the electron micrographs revealed a statistically significant increase in collagen fibril diameters in 3 month old double deficient mice versus 3 month old WT mice (Fig. 3), while there was no difference in collagen fibril diameters between 9 month old WT and double deficient mice.

**Gene Expression**

At three months, Real Time PCR gene expression analysis showed a statistically significant decrease in $PTHrp$ from double deficient mice compared to WT mice. At nine
months, we observed a statistically significant increase in ColX and Aggrecan from double deficient mice compared to WT mice (Fig. 4).

**Discussion**

In this study we examined microarchitectural and molecular changes in the subchondral bone and condylar cartilage before and during active TMJ-OA in a mouse model. Before overt TMJ-OA, we found a significant decrease in the expression of PTHrP in the mandibular condylar head from the Bgn/Fmod double deficient mice compared to our wildtype controls. In growth plate cartilages, PTHrP has been shown to keep the chondrocytes proliferating and to delay their further differentiation (Shibukawa, 2007). Therefore, the decrease of PTHrP at 3 months in the double deficient mice is consistent with our previous finding of a decrease in proliferation in the condylar cartilage from 3 month old male Bgn/Fmod double deficient mice (Wadhwa *et al.*, 2005).

During overt OA, we found a significant increase in the expression of Col X and Aggrecan in the mandibular condylar head from the double deficient mice compared to the WT controls. Increased expression of these molecules is consistent with an attempted repair response (Drissi *et al.*, 2005).

Transmission electron microscopy revealed an increase in the collagen fibril diameters in the condylar cartilage from the 3 month old double deficient compared to WT controls. Biglycan and fibromodulin are members of the small leucine repeat family (SLRP). Members of this family are thought to cause a reduction in the average collagen fibril diameters (Vogel and Trotter, 1987), which is consistent with our finding of an
increase in the collagen fibril diameters in the condylar cartilage in the absence of two SLRP family members. Changes in the collagen fibril diameters may cause changes in the mechanical properties of the condylar cartilage, modifying condylar cartilage differentiation (Pirttiniemi et al., 1996; Sasaguri et al., 1998) and making it more prone to degeneration (Hu et al., 2006). In the tendons of the double deficient mice, it was reported that there was a decrease in average collagen fibril diameters (Ameye et al., 2002), which suggests that the absence of biglycan and fibromodulin produce site specific effects. As the mice age there was an increase in collagen fibril diameter from the condylar cartilage between 3- and 9-month old WT mice. A similar age-related increase in the average width of the collagen fibrils has been reported in the TMJ disc from rats (Ahn et al., 2007), however, no difference was seen between 3-and 9-month double deficient mice. We have previously shown that the wildtype mice also develop TMJ-OA at 18 months. Therefore, the increase in the average diameters of the collagen fibrils from both the wildtype and the double deficient mice appears to be a pre-osteoarthritic biomarker of the condylar cartilage and suggest an accelerated TMJ-OA phenotype in the double deficient mice.

Controversy exists in the literature as to whether bone changes or cartilage changes occur first in osteoarthritis. Many believe that subchondral bone changes precede cartilage changes (Pastoureau, 1999), (Yamada, 2002). Micro-CT Analysis revealed no differences in any of the parameters examined in the mandibular subchondral bone between the double deficient and wildtype mice at 3 months. On the other hand, after overt OA lesions in the TMJ at 9 months, a general increased remodeling response was noted in the subchondral bone of the double deficient mice. Consequently, our data
suggest changes in the condylar cartilage precede changes in the subchondral bone in our TMJ-OA mouse model.

The exact etiology for TMJ-OA is unknown, however, most dentists and physicians have been inclined to believe that the single most important etiological factor is altered mechanical loading which surpasses the adaptive capacity of the joint (Milam, 2005; Zarb and Carlsson, 1999). In our study, we propose that the absence of biglycan and fibromodulin causes increased collagen fibril diameters of the condylar cartilage, which may alter the loading of the TMJ during normal masticatory function. In addition the decrease in PTHrP expression in the double deficient mice may influence the adaptive capacity of the joint. Greater understanding of the early molecular and structural changes during the initial stages of TMJ-OA are critical in order to develop new therapies in providing relief to patients who suffer from osteoarthritis of the temporomandibular joint.
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Figures

Figure 1. Representative images of the Safranin O staining (red) of the condylar cartilage. The slides were counter stained with Fast Green. A, 3 month Wildtype; B, 3 month Biglycan/Fibromodulin double deficient C, 9 month Wildtype D, 9 month Biglycan/Fibromodulin double deficient

Figure 2. Micro-CT analysis of the subchondral bone. Analysis of bone volume fraction (A), total volume (B), bone volume (C), trabecular thickness (D) and trabecular spacing (E) from the mandibular condylar subchondral bone of a total of 26 mice at 2 time points. At 3 months (n=6) in the wild-type group and (n=6) in the double deficient group. At 9 months (n=6) in the wild-type group and (n=8) in the double deficient group. Points are the mean and SEM for n=12 for the 3 month and n=14 for the 9 month groups. *Significant difference between wild-type and double-deficient (p<0.05).

Figure 3. Transmission Electron Microscopy analysis of the condylar cartilage. Analysis of collagen fibril diameters in (A) 3 month wild-type and (B) 3 month double-deficient mice (C) A graphic representation of the data comparing 3 month wild-type and double-deficient mice versus the 9 month wild-type and double-deficient mice. A total of 26 mice at 2 time points; at 3 months (n=6) in the wild-type group and (n=6) in the double deficient group. At 9 months (n=6) in the wild-type group and (n=8) in the double deficient group. Points are the mean and SEM for n=12 for the 3 month and n=14 for the
9month groups. *Significant difference between wild-type and double-deficient (p< 0.05).

Figure 4. Real Time PCR analysis for Parathyroid hormone related protein (Pthrp), SRY-box containing gene 9 (Sox9), Collagen type II (Col2), Indian hedgehog (Ihh), Collagen type X (Col10), Vascular endothelial growth factor (Vegf), Osteopontin (Opn), Osteoprotegerin (Opg), Collagen type 1 (Col1), Aggrecan (Aggrecan) and Runx-2 gene expression from the mandibular condylar head from (A), 3 month wild-type mice and 3 month double-deficient mice and, (B) 9month wild-type mice and 9 month double-deficient mice. A total of 45 mice were dissected for 2 time points. At 3 months n=10 in the wild-type and n=10 in the double-deficient group and at 9months n= 13 in the wild-type and n=12 in the double deficient group. Points are the mean and SEM for n=20 for the 3month and n=15 for the 9month groups. *Significant difference between wild-type and double deficient mice (p< 0.05).
References


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Discussion

Micro-CT

We compared the data in a multitude of combinations to detect any and all differences between the mice that developed osteoarthritis and those that did not. We first looked at the micro-CT data for 3-month-old mice—wild-type versus the double-deficient mice. As expected, there were no changes or differences in the subchondral bone between the double-deficient and wild-type mice. In comparison, when we begin to look at the 9-month data, we can first see differences appearing between the 9-month-old wild-type vs the biglycan/fibromodulin double-deficient mice. Even though we saw an increase in bone volume versus total volume, there was a statistically significant decrease in bone volume fraction, indicating that bone volume did not increase as much as the total overall volume.

X-ray Diffraction

We compared the difference in collagen fibril diameters at 3 months versus 9 months in the wild-type and double-deficient mice. There was a statistically significant increase in collagen fibril diameters in 3-month-old deficient mice compared to the controls. The increase in collagen fibril diameters could be attributed to pre-osteoarthritic changes that occur in the mice before they develop osteoarthritis. Xu et al. compared the collagen fibril diameters in wild-type and double-deficient mice. They showed an increase in collagen fibrils in the articular cartilage of knee joints from heterozygous chol+ mice compared to the wild-type mice [32]. The authors attribute this increase in collagen
fibril diameters to the ratio of collagen type II to collagen type X; there is a decrease in this ratio in the cho/+ mice thus causing this increase in collagen fibril diameter thickness. These structural changes that occur in the early stage in the double-deficient mice could be precursors to the TMJ-OA that develops in these mice with age as previously shown [23]. Although no significant differences were seen between the 9 month old wild-type and double deficient mice, interestingly the 9month wild-type were similar in collagen fibril diameter to the 3month double deficient mice. Since, even the wild-type mice develop osteoarthritis with age, it is not unforeseen that the 9month old wild-type mice would have similarities to the 3month old double deficient mice.

**Gene Expression**

Several studies of the knee joint have quantified gene expression through Real Time-PCR of the condylar cartilage of osteoarthritic animal models. In a sample of mature vs aged rabbits, with osteoarthritis induced with ACLT resection, it was shown that Fas, Caspase 8, and MMP-13 were elevated in the rabbits with induced OA [33]. In yet another study on dogs, the authors quantified genes that may be expressed in early versus late stage osteoarthritis. Early phase OA markers include col II, col I, and YKL40 and late stage markers include tenascinC and MMP-13 [34]. In the TMJ, Meng et al showed that in induced OA by means of alterations in the TMJ disc, rats show an increased expression of EGLN3, MMP3, AQP3, NOV, DKK3 [35].

In the murine model gene expression SRY box-containing gene (Sox-9) is increased, collagen type II (Col II) is increased [36], osteoprotegerin (Opg) is increased [37], vascular endothelial growth factor (Vegf) is increased [58], and collagen type X (Col X) is decreased [39].
The difference in the various studies mentioned previously is that all the models are different, some are naturally occurring and others are trauma-induced osteoarthritis. Moreover, there are several different animals that are used to study the disease progression which also makes it difficult to postulate broad assumptions. The disease process in the various studies also looks at the progression of the disease process under different time points which also makes it difficult to use for comparison, as many different markers vary with the course of the disease.

In our study we saw a decrease in \( \text{Pthrp} \) and \( \text{Osteocalcin} \) at 3 months in the double deficient mice compared to the controls. \( \text{Osteocalcin} \) is an important genetic marker present in both bone and cartilage. Since there is a decrease in \( \text{Osteocalcin} \) at 3 months and there are no significant changes in the bone, we propose that there are changes occurring in the cartilage. \( \text{Pthrp} \) is involved in an important negative feedback loop with Indian Hedgehog. As Indian Hedgehog increases, \( \text{Pthrp} \) is in a decreased level. \( \text{Pthrp} \) acts to keep chondrocytes in a proliferating stage; however, when \( \text{Pthrp} \) levels are low, there are less chondrocytes in the proliferation stage and more at the hypermaturation stages \[59\]. The decrease in \( \text{Pthrp} \) indicates that there is a decrease of cells in the proliferating stages and more cells in the mature state which is what we would expect to see in a diseased state of osteoarthritis. We saw a decrease of \( \text{Pthrp} \) at 3 months indicating that \( \text{Pthrp} \) may be a genetic marker in screening for pre-osteoarthritis.

At 3 months, we noticed a decrease in gene expression for Osteocalcin. \( \text{Osteocalcin} \) is highly expressed in bone, in growth plate cartilages \[60\] and is a marker for chondrocyte hypertrophy \[61\]. Possible explanations for the decrease in \( \text{osteocalcin} \)
expression in the 3 month old double deficient mice include a decrease in osteoblast differentiation, which has not yet manifested in changes in the micro architecture of the subchondral bone. Another possible explanation is that there is decreased terminal hypertrophic maturation in the mandibular condylar cartilage in the double deficient mice; however, other cartilage hypertrophic maturation markers (Col X, Vegf) were not reduced.

Available biomarkers delineating changes in the subchondral bone, fibrocartilage, or gene expression may be instrumental tools in pre-osteoarthritic screening. Since degenerative diseases cause irreversible damage and there are no known available biomarkers to detect the disease, any early biomarkers would be essential in diagnosing the disease to help alleviate symptoms or possibly to prevent further damage to the joint. Furthermore, early detection could lead to therapies that may be able to cure this debilitating disease process.

The TMJ-OA model is important in studying the entire disease process and poses possibilities for clinical relevance. We saw an increase in collagen fibril diameters, changes in biglycan and fibromodulin, and a decrease in PTHrP, all of which could lead to the development of osteoarthritis.

**Conclusion**

Based on our data, we believe that condylar cartilage changes precede subchondral bone changes. We showed an increase in collagen fibril diameter in the 3 month old double deficient mice and no significant changes in the 3 month old mice. We saw significant subchondral bone changes at 9 months, and altered gene expression at both 3 and 9 months.
Significance of Results

It is currently extremely difficult to study the progression of osteoarthritis of the temporomandibular joint especially at early stages. Limitations of studying osteoarthritis include difficulty in obtaining cross-sections of human studies and the absence of clinical signs or symptoms at early stages. Consequently, using animal studies is beneficial in studying disease processes of the tempopormandibular joint. With the advent of increased research, remedies can be sought to ameliorate the clinical symptoms. Understanding the role of the subchondral bone during TMJ-OA, may lead to new bone specific treatment modalities. Also, research of biglycan and fibromodulin can lead to new treatments that may be targeted at these specific proteoglycans in the temporomandibular joint in the prevention or cessation of disease progression of osteoarthritis.

Future Direction

It may be interesting to further study this project by:

1. Separating the female and male mice and examining changes that occur in the fibrocartilage and subchondral bone. Especially since temporomandibular disorders have a higher prevalence in females it may be beneficial to see if there are any pre-disposing factors in the female mice.

2. The mice deficient in biglycan/fibromodulin could be used in an altered mechanical loading model with incisor trimming of mice to study any condylar changes that may occur under a different mechanical enviornment. Since the temporomandibular joint has a limited capacity to regenerate, it may be beneficial to examine changes under an altered enviroment to examine any pre-disposing factors that lead to destruction of the joint.
Collagen Fibril Diameter

- **WT**
- **Double Deficient**

![Bar Graph](image)

**B** 3mWT

**C** 3mDouble Deficient

**200 nm**
References


