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Analysis of microarchitectural changes in a mouse temporomandibular joint osteoarthritis model

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ABSTRACT

Objective: Little is known about the natural progression of the disease process of temporomandibular joint (TMJ) osteoarthritis (OA), which affects approximately 1% of the US population. The goal of this study was to examine the early microarchitectural and molecular changes in the condylar cartilage and subchondral bone in *biglycan/fibromodulin* (*Bgn/Fmod*) double-deficient mice, which develop TMJ-OA at 6 months.

Methods: TMJs from 3-month-old ($n = 44$) and 9-month-old ($n = 52$) wild-type (WT $n = 46$) and *Bgn/Fmod* ($n = 50$) double-deficient mice were evaluated. Micro-CT analysis of the subchondral bone ($n = 24$), transmission electron microscopy for condylar cartilage fibril diameters ($n = 26$), and real-time PCR analysis for gene expression for bone and cartilage maturation markers ($n = 45$) was performed.

Results: A statistically significant increase in collagen fibril diameter of the condylar cartilage and a decrease in expression of *Parathyroid related protein* in the mandibular condylar head were observed in the 3-month *Bgn/Fmod* double-deficient mice compared to WT controls. The 9-month *Bgn/Fmod* double-deficient mouse demonstrated an increase in bone volume and total volume in subchondral bone, and an increase in the expression of *Collagen Type X* and *Aggrecan* in the mandibular condylar head compared to the WT controls.

Conclusion: We found that changes in the microarchitecture of the condylar cartilage preceded changes in the subchondral bone during OA in the TMJ in *Bgn/Fmod* double-deficient mice.

1. Introduction

The National Institute of Dental and Craniofacial Research of the National Institutes of Health reported that temporomandibular joint (TMJ) disease is the second most common musculoskeletal disease in the United States, with 10.8 million people suffering from TMJ problems at any given time. The TMJ is different than other joints in a number of ways.[1] Some of these differences are that the TMJ is composed of fibrocartilage instead of hyaline cartilage[2] and the mandibular

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Abbreviations: BGN, biglycan; FMOD, fibromodulin; TMJ, temporomandibular joint; OA, osteoarthritis.

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condylar cartilage undergoes endochondral ossification during the active growth period.[3] One form of TMJ disease is osteoarthritis (OA).[4] While imaging techniques are used to diagnose TMJ-OA, such diagnosis can only be made after the TMJ has been subjected to irreversible damage. Therefore, very little is known about the early stages of the disease process. With only a limited capacity for TMJ regeneration, greater understanding of the molecular and microarchitectural structural changes in the early stages of TMJ-OA is critical. Such research has been facilitated by the use of animal models to study the entire TMJ-OA disease process.[5]

Several animal models have been developed to study the effects of TMJ-OA. One such TMJ-OA model that covers the whole diseases process is the biglycan and fibromodulin (*Bgn/Fmod*) double-deficient mouse model. BGN and FMOD are members of the small leucine-rich protein family[6] essential in extracellular matrix organization in bone,[7] cartilage,[8] and tendons.[9,10] *Bgn/Fmod* double-deficient mice develop OA in a number of joints,[10] including the TMJ.[5] The *Bgn/Fmod* double-deficient mice also develop knee OA at 2 months, and by 5 months almost complete destruction of the joint occurs.[11] In this joint, structural weaknesses of tendons are thought to be the origin of OA.[10] In addition, other studies have shown that knee OA is associated with the articular chondrocytes undergoing endochondral ossification inappropriately.[12] In contrast, TMJ-OA is first detected at 6 months in the *Bgn/Fmod* double-deficient mice, and the disease progresses gradually with age.[5] In addition, both the origin of OA in the TMJ as well as whether reactivation of endochondral maturation during TMJ-OA occurs in the *Bgn/Fmod* double-deficient mice are unknown. Therefore, the goals of this study were to examine chondrocyte maturation markers and microarchitectural changes within the TMJ during early and active TMJ-OA. Greater understanding of such changes occurring in TMJ-OA samples is critical in the future development of treatment modalities that prevent or reverse the disease process.

2. Materials and methods

2.1. 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 WT mice were obtained from Jackson Laboratory (Bar Harbor, ME) and the double-deficient *Bgn/Fmod* mice in the B6/129 background were obtained from Dr. Marian Young (National Institute of Dental and Craniofacial Research, Bethesda, MD).[10] Both male and female mice ($n = 96$) were used in this study. Mice were euthanized at 3 or 9 months of age.

2.2. Histology

For each genotype and age, heads from at least three animals were dissected in two halves. After removal of the brain, the specimens were fixed for 2 weeks at room temperature in 10% formalin. After being washed in tap water for 5 min, they were decalcified in 15% EDTA for 1 week. Serial sections of the TMJ were then performed with every 5th section stained with H&E or Safranin O.[13]

2.3. 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). Mandibles from 3-month WT ($n = 6$), 3-month *Bgn/Fmod* double-deficient ($n = 5$), 9-month WT ($n = 6$), and 9-month *Bgn/Fmod* double-deficient ($n = 7$) mice were dissected and bisected at the level of the symphysis and stored in 70% ethanol. Analysis of the mandibular condylar head included the bone surface, bone volume, total volume, trabecular number, trabecular thickness, and trabecular spacing.

2.4. Transmission electron microscopy

Mandibles were dissected from 3-month WT ($n = 6$), 3-month *Bgn/Fmod* double-deficient ($n = 6$), 9-month WT ($n = 6$), and 9-month *Bgn/Fmod* double-deficient ($n = 8$) mice. The mandibles were bisected and placed in 2.5% glutaraldehyde/2.0% paraformaldehyde, buffered to a pH 7.3 with 0.1 M sodium cacodylate. The hemi-mandibles were placed in the fixative within minutes of sacrifice. The fixation proceeded for 24 h 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.[14] The condyles were removed from the hemi-mandibles and further demineralized for an additional 4 d, then rinsed in several changes of cold (4 °C) 0.1 M cacodylate buffer. Rinsed segments were postfixed with 1% osmium tetroxide in cacodylate buffer at room temperature for 2 h, dehydrated in a graded ethanol series and embedded in Polybed resin (Polysciences). The condyles were then sectioned sagittally at 1 μm 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.[15] The 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 52,000×. 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 10 collagen fibrils were chosen at random and their diameters measured using the measure tool. The examiner was blinded to the samples being measured.

2.5. RNA extraction and PCR amplification

The mandibular condyle was carefully isolated with the soft tissues removed using a dissecting microscope. Total RNA was obtained from the condylar head, which contained both