Unveiling 3D neurovascular dynamics in TMJ postnatal development and degeneration through whole joint mapping

Jiaxin Chai[1], Hanmei Dong[1], Benjamin Kheyfets[2], Wakana Kitagawa[2], Aidan Limpic[1], Dustin Mueller[3], Yaqian Sun[1], R. Glenn Hepfer[1,3], Brooke Damon[1], Tong Ye[1,3], Mildred Embree[1], Peng Chen[1,3], Hai Yao[1,3]
[1]Clemson University, Clemson, SC, [2]Columbia University, New York, NY, [3]Medical University of South Carolina, Charleston, SC
jchai@clemson.edu

Disclosures: None

INTRODUCTION: The temporomandibular joint (TMJ) is one of the most frequently used joints, and it plays essential roles in many necessary daily activities like eating, speaking and breathing. Temporomandibular disorders (TMDs) are a group of conditions that cause pain and dysfunction involving the masticatory muscles and TMJ. About 11-12 million adults in the United States suffer from pain in the TMJ region. Neurovascular networks are critical for maintaining joint health and hemostasis since vasculatures serve nutrient supply and biomolecule transport, while nerves serve pain sensation and neurotransmitter secretion. The neurovascular networks in the joint modulate the postnatal development of the joint. Besides, alterations of the neurovascular structure due to joint diseases will lead to changes in the joint environment, contributing to disease progression. Currently, there are only a few studies investigating the postnatal development of TMJ neurovascular networks in both human and animal models. Meanwhile, limited studies have investigated the effects of TMJ degeneration on the neurovascular structures, and existing findings in the literature are somewhat conflicting. Therefore, a comprehensive study of the 3D neurovascular networks during mouse postnatal growth and joint disease progression at the whole TMJ level is crucial to capturing development and disease features and investigating the underlying mechanisms. TMJ is a complex joint composed of multiple components with different tissue properties, making it very challenging to label and clear the whole TMJ using existing protocols. In this study, we used a whole joint clearing method that we recently developed and successfully mapped the 3D neurovascular structure in mouse TMJs with large-field light sheet imaging. With this advanced technology, we revealed the postnatal development of neurovascular networks in the TMJs of healthy mice and uncovered the time-course changes of neurovascular structures in a well-documented lubricant knockout (Prg4$^{-/-}$) joint degeneration mouse model.

METHODS: TMJs from C57BL/6 wildtype (WT) and Prg4$^{-/-}$ mice aged postnatal days 7 (P7, n=3), days 14 (P14, n=3), days 30 (P30, n=3) and days 90 (P90, n=3) were harvested freshly and immediately fixed in 10% neutral buffered formalin. Fixed TMJs were scanned with a micro-computed tomography (µCT) device, and the µCT dataset was imported to Imaris software for reconstruction and analysis. After decalcification, delipidation, decolorization, and deep permeabilization, the whole joint samples were immunostained with antibodies to CD31 for vessels and calcitonin gene-related peptide (CGRP) for pain-related nerves. Then, samples were cleared and imaged with a large-field light sheet microscope, and images were processed with Imaris. Given that TMDs are twice as common in women as in men, all mice used in this experiment were female. Studies on male mouse TMJs are ongoing.

RESULTS: The µCT results with WT mouse TMJs showed the changes in the mandibular condyle during healthy mouse growth, while with the data of Prg4$^{-/-}$ mouse TMJs, we observed altered growth patterns of the condyle in the diseased model from P14. Compared to the mandibular condyles of WT mice, the condylar heads from Prg4$^{-/-}$ mice displayed a flatter surface (Fig. 1A). Besides, our 3D mapping data of mice demonstrated the changes in the neurovascular distribution in TMJs during the growth of mice. From P7 to P30, we observed that the vascular distribution retreated toward the disc periphery. Compared to WT mice, the Prg4$^{-/-}$ mice showed different development patterns of neurovascular distributions in the TMJs, with a slower process of vascular regression in the anterior region of the disc. From P7 to P30, CGRP immunoreactive nerves appeared at the anterior, posterior, lateral and medial peripheral portions of the TMJ disc, but there were no CGRP+ nerves in the central region. In the TMJ discs from mice aged P30, we observed increased nerves and blood vessels in the anterior and posterior regions in Prg4$^{-/-}$ mice compared to WT mice (Fig. 1B). Through the 2D optical section from our 3D mapping data, we observed the TMJ disc thickening from P14 to P30 and found that at P30 the joint cavity enlarged (Fig. 1C).

DISCUSSION: This study comprehensively investigated the development of both hard and soft tissue in the whole TMJ across healthy and diseased mice. A previous study in mice with 2D histological analysis reported that at age 2 weeks, Prg4$^{-/-}$ mice exhibited a thickened TMJ articular disc, aligning with our findings. From P7, we found pain-related nerves distributed in the peripheral portion of the TMJ disc, with few nerves in the central region, and this is consistent with the results reported in the literature by 2D immunohistochemistry investigations in rats. Our 3D mapping of TMJs allows a more accurate description of the alterations of neurovascular networks during postnatal TMJ development and disease progression at a whole joint level. These results provide valuable 3D information for studying how neurovascular networks modulate postnatal development and disease progression of TMJ, and the interactions between nerves, vessels and joint structure. Ongoing studies are investigating sex-specific neurovascular development and disease variations, as well as the relationship between joint structure changes and neurovascular changes from the perspective of the whole TMJ.

SIGNIFICANCE/CLINICAL RELEVANCE: The 3D neurovascular mapping of the TMJ in healthy and joint-diseased mice at different ages can provide insights into the role of neurovascular networks in joint development and disease progression, which will advance our understanding of disease mechanisms and contribute to developing new treatment strategies for TMD.

ACKNOWLEDGEMENTS: This work is supported by funding from NIH: P20GM121342, R01DE021134, R34DE033593 and MTF Biologics.

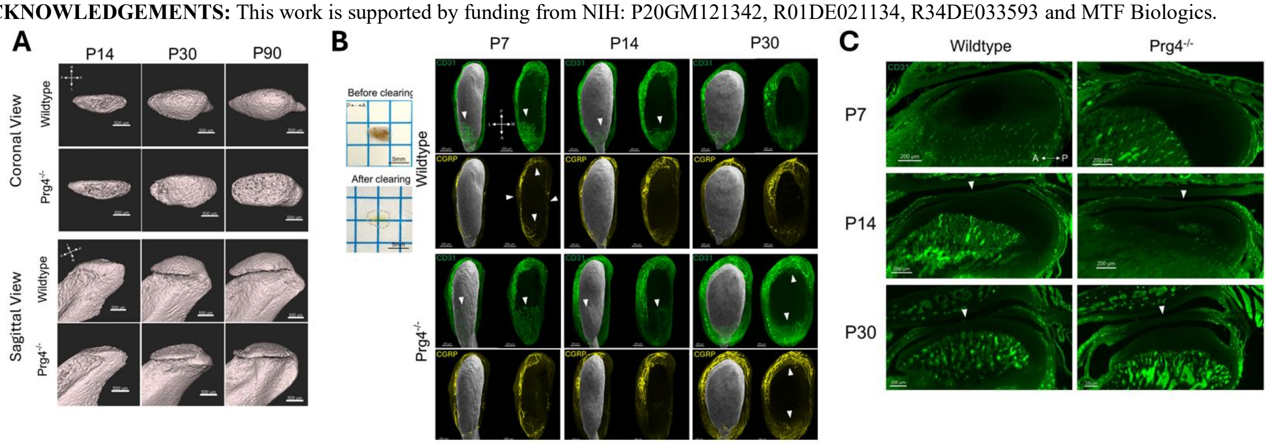

Figure 1. A. µCT reconstruction of mouse TMJ condylar heads in wildtype and Prg4$^{-/-}$ mice aged postnatal 14 days, 30 days and 90 days. **B.** 3D TMJ neurovascular mapping in wildtype and Prg4$^{-/-}$ mice aged P7, P14 and P30. White arrows highlight the regions with neurovascular structure changes. **C.** 2D optical section from 3D TMJ neurovascular mapping in wildtype and Prg4-/- mice aged P7, P14 and P30. White arrows highlight TMJ disc differences.