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AAO Foundation Final Report Form
(a/o 6/30/2020)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award:
-- Biomedical Research Award

Name(s) of Principal Investigator(s)
--Jing Chen

Institution
--Columbia University

Title of Project
--Role of Alpha-2 Macroglobulin (A2M) Signaling Pathway in Mediating TMJ Degeneration

Period of AAOF Support (e.g. 07-01-20 to 06-30-21):
--originally 07-01-2018 to 06-30-2019, extended once to 12-31-2019.

Amount of Funding
--$30,000

Summary/Abstract
Alpha-2 Macroglobulin (A2M) is a plasma protein found in blood. It is produced in liver, and Acts as an antiprotease and inactivates a large variety of proteinases.We have found that one of the most significantly mediated genes in the TMJ by estrogen replacement in Ovx wild type mice

was Alpha 2 Macroglobulin (A2M), which was confirmed by q-PCR analysis of A2M gene expression in a separate, independent experiment. We have further found that A2M dose dependently inhibited degradation of collagen type 1 and 2 from adult WT mandibular condylar cartilage organ cultures. A2M has been shown to inhibit the progression of surgically induced knee osteoarthritis by a unique bait and trap mechanism that is able to sequester cytokines and proteases, inhibiting their activity. However, the role of A2M in mediating TMJ-DJD remains unknown. Therefore, our hypothesis for this project is that A2M inhibits protease activity protecting the TMJ from degeneration.
Our study examined the role of A2M in regulating IL-1B induced inflammation in the condylar cartilage. Treatment with IL-1B in female mice caused a statistically significant increase in collagen type 1 and type 2 cleavage epitopes, MMP-3 gene expression, and MMP-9 gene expression. Addition of A2M in the presence of IL-1B caused a decrease in collagen type 1 and type 2 cleavage epitopes, MMP-3 gene expression, and MMP-9 gene expression. These results support the findings of past studies that correlate A2M with a reduction in protease expression and collagen type 1 and 2 degradation. A2M inhibits IL-1B TMJ degeneration. Interestingly, there was no significant difference found between the control groups and A2M groups. It would have been expected that addition of A2M would result in less TMJ degradation than controls, however the data suggests that A2M is beneficial only in a state of degeneration. The results of the study show that A2M inhibits IL-1B induced TMJ degeneration and IL-1B induces TMJ degeneration.

Detailed results and inferences:

1. If the work has been published, please attach a pdf of manuscript
   It has not been published.

2. Describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis and interpretation of results should be included.

Delineate the role of A2M in regulating IL-1 induced inflammation in the condylar cartilage
Hypothesis –A2M inhibits the level of inflammation caused by inflammatory factor IL-1.
Investigated: Organ culture of mandibles from female WT mice has been conducted with treatment with vehicle, A2M 100nM, A2M 200nM, IL-1β (10 ng/ml), IL-1β (10 ng/ml) + A2M (100nM) and IL-1β (10 ng/ml) + A2M (200nM). Real-time PCR on condylar cartilage showed A2M (200nM) decrease protease expression, IL-1β can increase expression of proteases. The increased expression of protease induced by IL-1β can be inhibited by addition of A2M (200nM). It was decided to use A2M 200nM as the concentration for the following experiment.

Results:
Collagen Type 1 (C1) and 2 (C2) Cleavage ELISA assay showed decreased C1C2 concentration on A2M (200nM) treatment, and an increase on IL-1β treatment. A2M + IL-1β treatment showed a reduction of C1C2 concentration from IL-1β treatment, indicating the addition of A2M rescued the collagen cleavage induced by IL-1β. There was no significant difference between the control and the A2M + IL-1B group. (Figure 1)

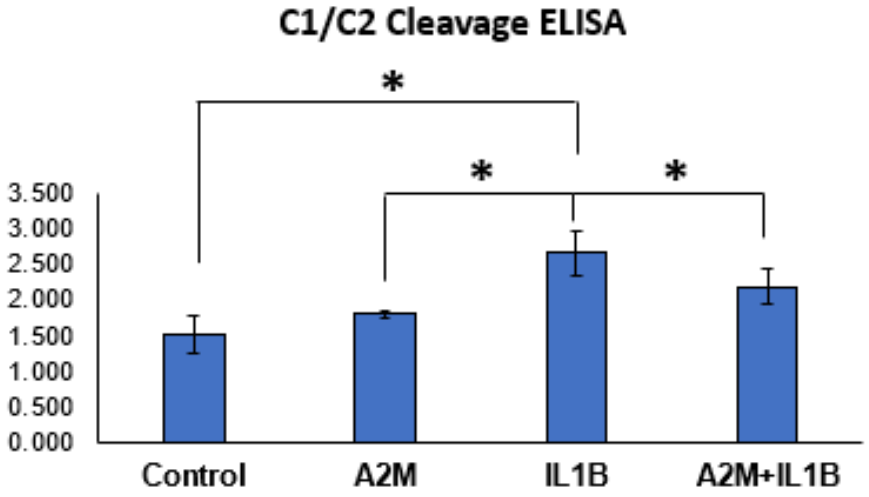

Figure 1. Female Mice - Collagen Type 1 and Type 2 cleavage fragments as measured by ELISA

Real-time PCR was performed with the RNA extracted from the condyle cartilage with these four treatment.
In the experiments of female mouse condyle culture, IL-1β treatment significantly increased the expression of MMP3 and MMP9 (Figure 2). Addition of A2M to the IL-1β treatment decreased the level of MMP3 and MMP9 expression similar to the control.

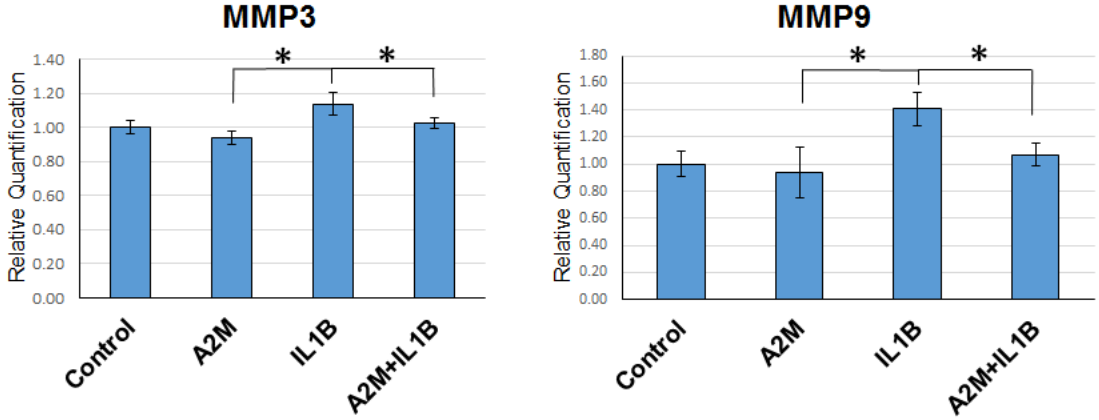

Figure 2. Female Mice. MMP-3 and MMp-9 gene expression.

In the experiment of male mouse condyle culture, there was no significant difference between the groups (Figure 3). There was a trend for an increase in MMP-3 and MMP-9 with the addition of IL-1B. There was also a trend towards less MMP-3 and MMP-9 expression with the addition of A2M and IL-1B when compared to the IL-1B only group. These trends mirror the significant differences found in female mice for MMP-3 and MMP-9 expression.

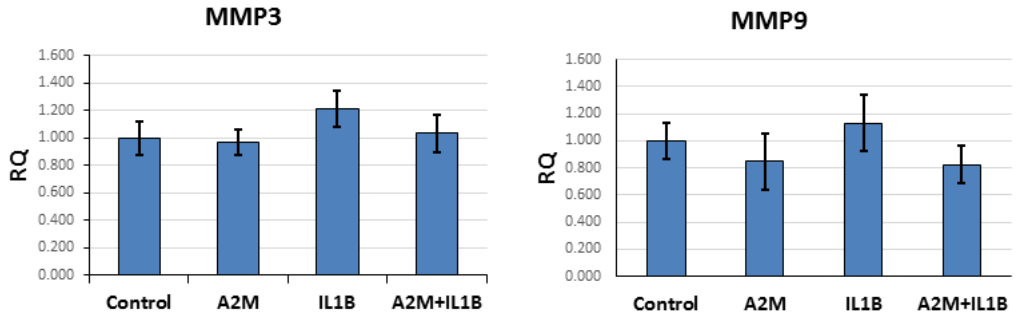

Figure 3. Male Mice. MMP-3 and MMp-9 gene expression.

Aim 2. Determine the efficacy of TMJ intra-articular injection of A2M for the treatment of TMJ-DJD

Hypothesis- Intra-articular TMJ injections of A2M will inhibit chemically induced TMJ osteoarthritis by inhibiting protease activity.

Experimental design-Intra-articular TMJ injections of A2M will be performed in male and female rats that are exposed to chemical induced osteoarthritis. TMJ degeneration and function will be analyzed.

Investigated:

C57B/L6 male mice were tried with injection of MIA instead of rats, with 0.25mg MIA in 8ul solution for each joint. The injection is required to be accurately into the lower chamber of the TMJ capsule. However, the preliminary histological results indicated a non-consistency on the damage of condyle cartilage. Some sections show the damage in condyle, some show no different from the control injected with saline only. This may related to the maneuver difficulty of injection. This inconsistency result with MIA only makes it almost impossible to evaluate the possible treatment effect of A2M.

A modification on the experiment method has been tried to create a reliable osteoarthritis model and reliable experiment result. A test with exposure TMJ for injection was conducted. However, the morbidity of TMJ exposure was high-only 1 or 2 mice out of 8 mice in each group survived from initial TMJ exposure. And these survived mice were later eliminated from experiment due to significant loss of body weight. None of these mice survived from second TMJ exposure.

<u>Respond to the following questions</u>:

1. Were the original, specific aims of the proposal realized?

   Aim 1. Delineate the role of A2M in regulating IL-1 induced inflammation in the condylar cartilage—realized

   Aim 2. Determine the efficacy of TMJ intra-articular injection of A2M for the treatment of TMJ-DJD—was not realized, due to the problems with experiment maneuver.

   I intended to have one or more trials on in vivo experiments. However, the experiment had to be abolished due to COVID shut down. As of now, due to COVID-related uncertainty and exposure risk, there is a shortage on clinic faculty supervision. I have to fully involved in teaching and clinic supervision. The continuation of the project is not

expected to be resumed in a short time.
New Update-
A small trial with 3 mice in each group was conducted after the report of Aug 2020. The problems with high morbidity and high variability of the results made it impossible to draw a conclusive result.
The experiment with mice model was terminated. An in vivo experiment with larger animal model (such as rats) is suggested for the future exploration on this project.

2. Were the results published?-NO.
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers
    b. Was AAOF support acknowledged?
    c. If not, are there plans to publish? If not, why not?
    We are expecting to have some substantial results for Aim 2 for publication.

3. Have the results of this proposal been presented? -YES
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
    The initial result was presented at 2019 Annual Meeting of North Atlantic Component of Edward H. Angle Socity of Orthodontists.
    Title: Role of Estrogen Receptors in Mediating TMJ Growth and Remodeling
    Co-authors: Jing Chen, Christopher Ricupero, Bianca Cabri, and Sunil Wadhwa
    Year: 2019
    Location: Rochester, NY

    The research was also presented at NESO 2019 meeting in Boston.
    Title: The role of alpha 2 macroglobulin in mediating TMJ-DJD
    Co-authors: Jing Chen, Christopher Ricupero, Bianca Cabri, and Sunil Wadhwa
    Year: 2019
    Location: Boston, MA

    b. Was AAOF support acknowledged?
    YES.

    c. If not, are there plans to do so? If not, why not?

3. To what extent have you used, or how do you intend to use, AAOF funding to further your career?
    The award from the Foundation provides invaluable support for professional and career development. The AAO Foundation award provides necessary funds for me to start an independent research project, by collecting pilot data as basis for future research funding.

Accounting for Project: i.e., any leftover funds, etc.

Row Labels	FY19 Updated Budget	FY19 Actual	FY20 Budget	FY20 update	2/29/2020	Remaining Budgeted Balance	
GT002518 ORTHODONTIC RES	**30,000**	**12,844**	**0**	**17,155**	**17,294**	**-139**	Chen AAOF
61500 Lab Supplies	6,000	8,168		8,000	8,196		
64005 Services General (Chen)	7,840			5,000	5,000		
64335 MEMBERSHIP COSTS	2,000						
65205 DOMESTIC TRAVEL-GENERAL		1,934		0	609		Angle, NESO
65601 ICM Acquisitions	11,440	2,367		4,155	1,614		Chen AAOF.
65613 ICM per diem	2,720				1,550		
64110 LAB SERVICES		375			325		