Sex-dependent spinal expression of *SERPINA1*/Alpha-1 antitrypsin may predispose women to inflammation

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SIGNIFICANCE/CLINICAL RELEVANCE: Low expression of spinal alpha-1 antitrypsin (AAT-1) may partially account for the heightened frequency of inflammation and back pain in women.

INTRODUCTION: Women exhibit stronger inflammation-driven pain responses compared to men, suggesting a distinct interplay between sex and inflammation; potentially indicating a need for unique treatment options for women[(1)]. Several bulk- and single-cell bioinformatics approaches[(2,3)] have identified *SERPINA1* (human)/ *serpinA1a-e* (mouse) as a prominent gene in intervertebral disc (IVD) cells during IVD degeneration, but the role of *SERPINA1* in the IVD and adjacent bone are unclear. *SERPINA1* (SERine Proteinase INhibitor A1) is a gene that encodes AAT-1 protein, which suppresses recruitment of myeloid-derived cells (macrophages, neutrophils) to the site adjacent of injury[(4)] and is a protease inhibitor of elastase. AAT-1 deficiency exhibits a greater occurrence and prevalence in males, yet it is associated with a lower mortality rate compared to females[(5)]. We find by RNA-sequencing that IVD injury activates more inflammatory pathways in female mice than male mice (unpublished). Therefore, we hypothesized that *SERPINA1*/AAT-1 is differentially expressed in spinal tissues/cells by sex of mice and humans.

METHODS: This study is approved by IACUC. Tissues (liver, lungs, IVD, bone) were harvested from 4-month-old male and female mice (n=5/sex). The tissues were subsequently pulverized, and processed into mRNA, which was subsequently converted into cDNA to determine *serpinA1a* gene expression by qPCR. Lumbar L1-L3 was used for histological staining of SafraninO (IVD degeneration score) and AAT-1(immunofluorescent), L3-L5 IVDs for gene expression, L6-S1 for IVD mechanics and L6 vertebra for µCT. Bone marrow was extracted from tibia, femur, and illium of C57BL6 mice and used as a cell source for osteoclast induction. While M-CSF facilitated the differentiation of marrow cells into macrophages, the combination of M-CSF+RANKL promoted osteoclast formation, and M-CSF+RANKL+IL-1β supported both osteoclast formation and differentiation[(6)]. Ten human IVDs from male (n=5) and female (n=5) individuals within the age range of 28-85 years, were collected. The IVDs were categorized based on their degeneration level using the Thompson grading system, ranging from 1 (healthy) to 5 (most degenerated). Subsequently, these IVDs were processed and stained for AAT-1. A 2-way ANOVA was used to compare *serpinA1a* expression by tissue and sex or osteoclast expression of *serpinA1a* expression by osteoclast-differentiation by treatment (RANKL, RANKL+IL-1β) and sex. A Student's t-test compared sex for all other comparisons, with statistical significance defined at a $p<0.05$.

RESULTS: *SerpinA1a* expression is differentially expressed across mouse tissues, where the order from most to least expression of the tissues harvested is liver, lung, IVD and bone (**Fig. 1A**). Further, female tissues expressed less *serpinA1a* than males in lung, IVD and bone cells (mostly osteocytes) by 6-fold, 4-fold and 2-fold, respectively. Compared to male murine IVDs, females expressed 97% less AAT-1 protein (green color) in the IVD, particularly annulus fibrosus (AF) cells (**Fig. 1B, B'**). Next, we focused on bone marrow-derived osteoclast differentiation because the histological expression of AAT1 was intense in the bone marrow of vertebrae (data not shown). In males, treatment to induce osteoclast differentiation (RANKL or RANKL+IL-1β) reduced *serpinA1a* gene expression by ~0.4 fold but, in females, the treatment-induced osteoclast differentiation inconsistently reduced *serpinA1a* expression (interaction: $p<0.05$) (**Fig. 1C**). In human IVD, the AAT-1 protein expression in male IVD is less than that of women by 76% (**Fig.1D, D'**). Investigation of the remaining outcomes is currently ongoing.

DISCUSSION: Males consistently exhibited higher *serpinA1a*/AAT-1 expression levels than females in various tissues. Notably, the annulus fibrosus of the IVD and bones expressed less *serpinA1a/AAT* among all tissues tested, which could promote greater recruitment of neutrophils and macrophages as occurs in the lungs of people with a genetic variant in SERPINA1[(7)]. In human IVDs, AAT1 protein expression was greater in male IVDs than female IVDs, highlighting a conserved sexual dimorphism across species. In bone, we harvested a predominately osteocyte-rich tissue for qPCR and the sexual disparity in the expression of *serpinA1a* was mild. However, the expression of *serpinA1a* with osteoclast differentiation was strikingly different between biological sex. In myeloid-derived osteoclasts, RANKL promotes osteoclast differentiation and the presence of RANKL downregulated *serpinA1a* in males and the inclusion of IL-1β further promoted differentiation and suppression of *serpinA1a*. By contrast in bone marrow-derived female macrophages, RANKL appeared to upregulate *serpinA1a* during osteoclastic differentiation and IL-1β downregulated *serpinA1a* with further differentiation. Overall, the sexual dimorphic expression of *serpinA1a* in the spine and regulation of *serpinA1a* during osteoclast differentiation suggest that inflammatory signaling may yield greater inflammatory-related consequences to the musculoskeletal system of women than men.

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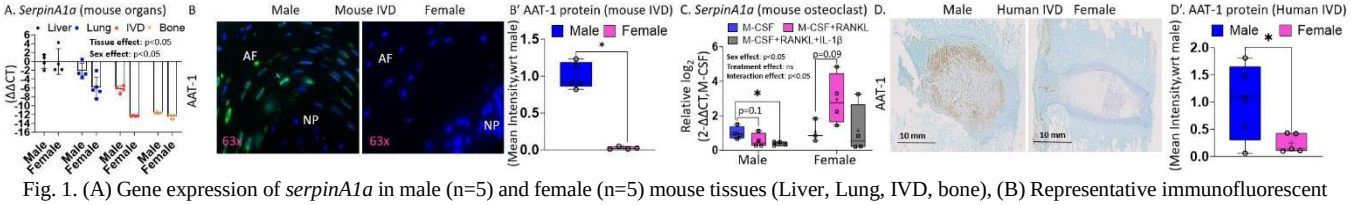

Fig. 1. (A) Gene expression of *serpinA1a* in male (n=5) and female (n=5) mouse tissues (Liver, Lung, IVD, bone), (B) Representative immunofluorescent images of AAT-1 (magnification 63x) in CON male (n=5) and female (n=5) IVDs. Blue color (DAPI) represents cell nuclei while green is the AAT-1 (B') Quantification of AAT-1 protein in the mouse male (n=5) and female (n=5) IVD, (C) Gene expression of *serpinA1a* in the macrophages (M-CSF), pre-osteoclast (M-CSF+RANKL) and osteoclast (M-CSF+RANKL+IL-1β) (n=4/group), (D) Representative immunohistochemistry images of AAT-1 in the human male and female IVD, (D') AAT-1 protein expression in the human IVDs of male and female (N=5/sex), *: $p<0.05$, ns-not significant