SerpinA1a Deletion in Aggrecan-Expressing Cells Reduced Vertebral Endplate and Tibial Subchondral Bone Volume

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Introduction: The vertebral endplate (EP) and tibial subchondral bone share a specialized architecture that integrates cartilage with bone, enabling load transfer, nutrient diffusion, and tissue homeostasis [1]. In the spine, EP erosion is strongly implicated in the onset and progression of intervertebral disc (IVD) degeneration (IVDD), a major contributor to back pain [2]. Similarly, structural and compositional changes in subchondral bone are key features of osteoarthritis, contributing to cartilage degeneration and impaired joint mechanics [3]. Alpha-1 antitrypsin deficiency (AATD), resulting from mutations in the *SERPINA1* gene, exacerbates neutrophil recruitment to the liver and lungs and drives elastin degradation, while also increasing the risk of osteoporosis and IVDD [4,5]. Despite these associations, the skeletal mechanisms underlying AATD remain poorly understood. Mice carry five *SerpinA1* paralogs and our unpublished data identified *SerpinA1a* as the most significantly downregulated paralog following IVD injury in a genetic mouse model of aged IVD cells. This finding led us to develop a conditional *SerpinA1a* knockout in aggrecan-expressing cells to investigate the musculoskeletal consequences of chondrocyte-specific deletion. We hypothesized that deletion of *SerpinA1a* in aggrecan-expressing cells of skeletally mature mice would not affect vertebral EP or tibial subchondral bone structure or density.

Methods: To model symptomatic AATD, which typically manifests in middle-age, 12-month-old *SerpinA1a* conditional knockout (*SerpinA1a*$^{fl/fl}$;*Aggrecan*^{CreERT2}, cKO) and wildtype (*SerpinA1a*$^{fl/fl}$, WT) male and female mice were fed tamoxifen chow *ad libitum* for 4 days to induce deletion of *SerpinA1a* in aggrecan-expressing cells in IACUC-approved experiments. Mice were euthanized and imaged by microCT at 13 months of age. The superior vertebral EPs of L6 and CC7 and the proximal tibial subchondral bone were analyzed for bone volume (BV), bone volume fraction (BV/TV), and volumetric bone density (vBMD). Measurements were compared to age- and sex-matched WT controls (n=3-10). Group differences were assessed using a two-way ANOVA, with a p-value < 0.05 considered significant.

Results: Conditional deletion of *SerpinA1a* in aggrecan-expressing cells significantly reduced trabecular (Tb) BV within the coccygeal EP (-9.8%)(**Fig. 1B'**) based on a genotype effect (two-way ANOVA, sexes pooled), with no corresponding change observed in the lumbar EP (**Fig. 1A'**); BV/TV remained unaltered in both regions. Similarly, a significant genotype effect (two-way ANOVA) was observed for total Tb BV, with reductions in both lumbar (L6; -15.5%)(**Fig. 1C'**) and coccygeal (CC7; -30%)(**Fig. 1D'**) vertebrae compared to WT controls, without changes in BV/TV or vBMD. In the subchondral region of the proximal tibia, *SerpinA1a* deletion likewise reduced total Tb BV (-11.5%)(**Fig. 1E'**) without impacting BV/TV or vBMD.

Discussion: Although no changes in bone structure were anticipated following cartilage-specific deletion of *SerpinA1a*, our findings of reduced BV in lumbar and coccygeal vertebrae—particularly within the coccygeal EP and tibial subchondral bone—suggest the mechanisms of bone structural loss may be derived from loss of *SERPINA1* in chondrocytes. One possibility is that the reduction in BV reflects enhanced neutrophil recruitment to cartilage-rich regions neighboring bone, contributing to local inflammation and bone loss [6]. Another possibility is that early *SerpinA1a* deletion due to the unintended 'leakiness' of the Aggrecan-Cre driver [7] targeted growth plate and/or EP chondrocytes (or other aggrecan-expressing cell types) during development, impairing bone growth [8]. Such disruption is consistent with unpublished baseline behavioral differences of impaired gait, potentially derived from structural abnormalities in the knee and spine. Given that *SERPINA1* is abundantly expressed in human chondrocytes and upregulated during chondrogenic differentiation [9], its absence during skeletal maturation and/or excessive neutrophil recruitment may contribute to AATD-induced osteoporosis in individuals with a *SERPINA1* variant.

Significance/Clinical relevance: These findings suggest that osteoporosis in AATD patients with SERPINA1 variants may derive from, or be influenced by, *SERPINA1* expression in chondrocytes, revealing a novel lung-independent therapeutic target.

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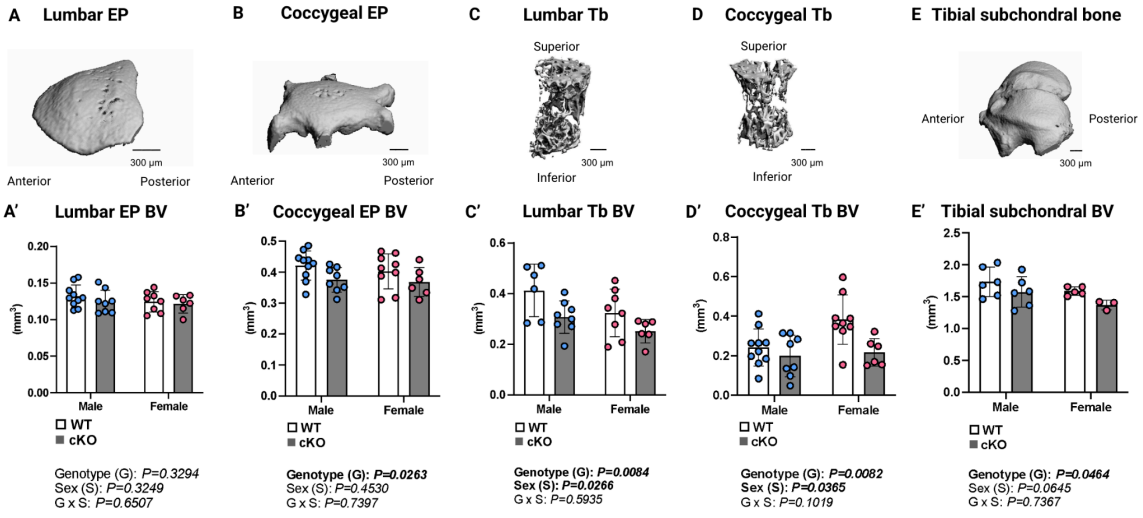

Figure 1: 3D reconstructions and quantification of BV of L6 EP (A, A'), CC7 EP (B, B'), L6 Tb bone (C, C'), CC7 Tb bone (D, D') and tibial subchondral bone (E, E') of *SerpinA1a* cKO and WT male (blue circles) and female (pink circles) mice. Scale: 300 µm. BV: bone volume, EP: endplate, Tb: trabecular.