Rare variants of *SERPINA1* alter spinal curvature in men and raloxifene partially restores spinal structure in male serpinA1ac KO mice

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SIGNIFICANCE/ CLINICAL RELEVANCE: Spinal treatment guidelines do not exist for chronic obstructive pulmonary disease (COPD) patients [1] with a variant in *SERPINA1* because the relationship between variants in *SERPINA1* and spinal structure is unclear.

INTRODUCTION: Deficiency of alpha-1 antitrypsin (AAT) from variants in gene precursor *SERPINA1* exacerbates chronic obstructive pulmonary disease [2] by excessive degradation of elastin and, for unknown reasons, is associated with back pain [3] and an elevated risk of vertebral skeletal fracture [4]. *SERPINA1*/AAT has multiple functions and variants in *SERPINA1* may promote intervertebral disc (IVD) dysfunction and bone loss. IVD-related mechanism: AAT inhibits neutrophil and/or macrophage elastase by reducing cellular chemotaxis and suppressing cellular adhesion to endothelial cells [5], and variants of *SERPINA1* may promote elastin degradation. Bone-related mechanism: AAT-deficiency increases the risk of osteoporosis and injection of human AAT to ovariectomized mice partially attenuates osteoclast-mediated resorption [6]. Raloxifene hydrocholoride (RAL) is an FDA-approved selective estrogen receptor modulator (SERM) that promotes bone accrual by inhibiting osteoclast-mediated bone resorption and we find that raloxifene stimulates estrogen signaling in the IVD to augment structure [7]. We hypothesize (Study #1) that variants of *SERPINA1* will alter the spinal curvature of the human spine from structural changes to the IVD and/or vertebral bone and (Study #2) that raloxifene injections will restore the structural properties of the IVD and vertebral bone in a mouse model of *SERPINA1* deletion.

METHODS: Study 1: From the SPIROMICS study [2], we acquired 2,168 CT scans of the thoracic region for women (n=1,077) and men (n=1,091) with COPD (GOLD spirometric grades 1-4) and without COPD and we acquired the DNA sequencing for the variants of *SERPINA1* in the patients. Using the Cobb's angle method (**Fig. 1A)**, we determined the Kyphosis angles between T3 and T11 for 288 patients thus far (MM (control): n=65, MV (any rare variants): n=45, MS: n=112, MZ: n=55, VV: n=1, ZV: n=2, ZS: n=2, ZZ: n=6) patients. Groups were compared by t-tests and significance set $p<0.05$.

Study 2: L1-L3 motions segments were harvested from CRISPR/Cas9-generated [8] 4-month-old male and female serpinA1a_c KO and WT mice (n=4-5/sex/genotype). Motion segments were stained for Safranin-O/Fast-green for histological IVD degeneration scoring and measured for IVD height. Next, 10-week-old male WT and serpinA1a-c KO mice were SQ injected with either PBS (Vehicle) or raloxifene hydrochloride (SIGMA) (n=4-5/genotype/treatment) at 0.5mg/kg 5x/week for 6 weeks. At 16 weeks of age, L1-L3 motions segments were harvested for histology and L3-L5 IVD were harvested for qPCR of *estrogen receptor alpha (er-α), aggrecan* and *18s* gene expression. Spines were *in vivo* scanned by micro-CT at 10- and 16-weeks of age at a resolution of 8µm. A protected 2-way ANOVA was used to determine sex, genotype or treatment effects; where a significant interaction would allow a post hoc test.

RESULTS: Study 1: In MM patients, Kyphosis angle was not different between males and females, whereas Kyphosis angle in MV patients was less in males than females by 18% (**Fig. 1B**). Study 2: *SerpinA1ac* deletion in mice corroborated the sexual dimorphism observed in humans where males exhibited 24% less IVD height (**Fig. 1C**), 47% higher IVD degeneration scores (*data not shown*) and 50% less new Tb.BV/TV accrual (**Fig. 1E**). SerpinA1ac deletion downregulated *ER-α* expression by 2-fold and therefore we treated WT and SerpinA1ac KO mice with estrogen agonist raloxifene. In WT and serpinA1ac KO IVDs, raloxifene upregulated *er-α* expression by 10-fold and *aggrecan* expression by 3.5-fold (**Fig. 1D**). Injection of raloxifene did not increase Tb.BV/TV in male WT nor serpinA1ac KO vertebrae (**Fig. 1E**). At 10wks, Tb.BV/TV was not different between vehicle and raloxifene-treated mice.

DISCUSSION: The Kyphosis angle of men was less than of women with any rare variant (MV) and this sexual dimorphism in spinal curvature corroborates the delayed detection of symptomatic COPD from AAT-deficiency in women than men [9]. Altered kyphosis angles can occur from bone loss or IVD degeneration [10] and can be associated with low back pain [11]. Using a mouse model of COPD [8], we found that IVD degeneration score, IVD height and bone structure were sex-dependent, with males KOs demonstrating a detrimental structural phenotype. Therefore, we proceeded with injecting raloxifene to male mice to counter the spinal changes because *serpinA1ac* deletion downregulated the expression of *estrogen receptor alpha* expression. Similar to the effect in WT mice [7], raloxifene normalized the IVD degeneration score of the *serpinA1ac* KO IVD and upregulated *aggrecan* expression. However, we and others find that raloxifene injection does not affect the trabecular nor the cortical vertebral bone structure in male mice, but this indicates that the benefit of raloxifene to the IVD may not necessarily be through crosstalk with bone. Overall, these data suggest that variants of *SERPINA1* affect the spine and that bone therapeutics may be repurposed to treat the musculoskeletal dysfunction in patients with AAT-deficiency-induced COPD.

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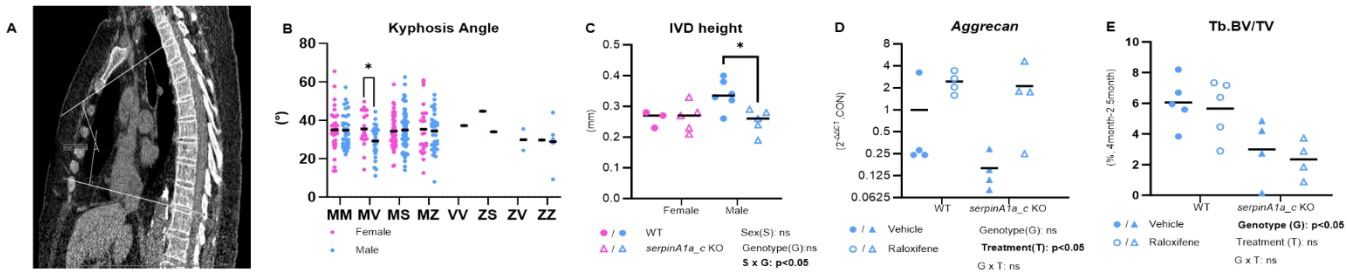

Figure 1. (A, B) Kyphosis angle for patients with *SERPINA1* variants and without (MM). (C) Intervertebral disc (IVD) height of male and female WT and serpinA1a_c KO mice. (D) *Aggrecan* gene expression and (E) Tb.BV/TV of male WT and serpinA1a_c KO mice treated with vehicle or raloxifene. Dark lines represent the average of the data.