Variants associated with adolescent idiopathic scoliosis perturb an estrogen-sensitive *Pax1-Col11a1-Mmp3* signaling axis

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Adolescent idiopathic scoliosis (AIS) is a common and progressive spinal deformity that exhibits striking sexual dimorphism, with girls at more than five-fold greater risk of severe disease

compared to boys. Despite its medical significance, insights into the pathogenesis of AIS are just emerging. By genome-wide association and functional studies we previously defined a female-specific risk locus in an enhancer near the *PAX1* gene. Here we sought to define the roles of *PAX1* and newly-identified AIS-associated genes in the mechanism of AIS. In a discovery and follow-up meta-analysis association study of nonsynonymous variants within extracellular matrix (ECM) genes (total N=103,757 individuals), we identified significant associations with a variant in *COL11A1* (rs3753841; p.(Pro1335Leu); $P=7.07e^{-11}$, OR=1.118). Using CRISPR mutagenesis we generated *Pax1* knockout mice (*Pax1*$^{-/-}$), which were viable and displayed a kinked tail phenotype. By RT-PCR in tails of E12.5 *Pax1*$^{-/-}$ mice we found reduced expression of AIS-associated genes including *Col11a1*. Immunofluorescence microscopy in postnatal spines detected overlapping staining of Pax1 and Col11a1 at the cartilaginous endplate-osseous junction encompassing the vertebral growth plate, with reduced expression of *Col11a1* in *Pax1*$^{-/-}$ spines compared to wildtype. To study the role of *Col11a1* in growth plate cells (GPCs), primary rib cartilage from *Col11a1*$^{fl/fl}$ mice was cultured in the presence or absence of Cre-expressing adenovirus. By RT-PCR in these cells we observed significant upregulation (P<.05) of *Mmp3,* encoding the matrix metalloproteinase 3 "stromolysin" enzyme that is known to be regulated by *Col11a1* in solid tumors. Conversely, endogenous *Mmp3* expression was significantly downregulated after lentiviral overexpression of the human *COL11A1*^{WT}, but not *COL11A1*^{P1335L}, in Cre-expressing *Col11a1*$^{fl/fl}$ SV40-immortalized GPCs. These results support negative regulation of *Mmp3* expression by *Col11a1* that is abrogated by the AIS-associated *COL11A1*^{P1335L} variant in GPCs. *Col11a1* is regulated by estrogen receptor beta (*ESR2*) in ovarian cells. siRNA-mediated *Esr2* knockdown in mouse GPCs significantly increased *Col11a1* expression, and significantly decreased *Mmp3* expression, as did tamoxifen treatment of these cells. These studies support a new model wherein genetic variation and estrogen signaling increase

susceptibility to spinal deformity during adolescent growth via a *Pax1-Col11a1-Mmp3* signaling axis.