Interactions Between ADAMTS10 and ADAMTS17 Regulate Growth Plate Function and Bone Growth

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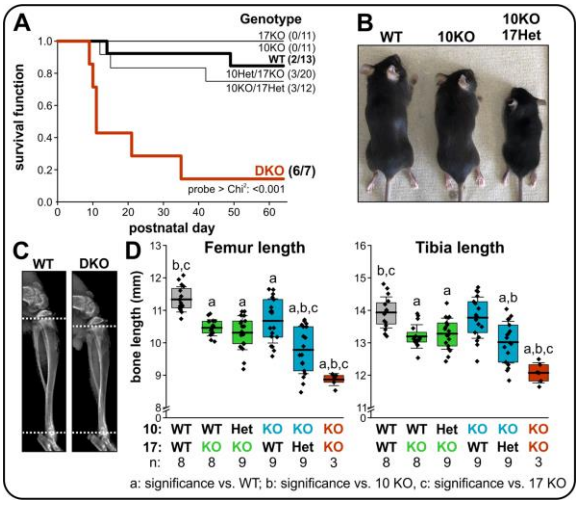
INTRODUCTION: Mutations in the secreted extracellular matrix (ECM) proteins ADAMTS10 and ADAMTS17 cause Weill-Marchesani syndrome (WMS)[1]. WMS is characterized by severe short stature, brachydactyly, joint stiffness, pseudomuscularity, thick skin, lens dislocation, and heart valve anomalies. Hence, these experiments of nature provide unique opportunities to reveal the functions of ADAMTS10 and ADAMTS17 in regulating human height and in regulating the homeostasis of other tissues affected in WMS. Human height is by and large the result of bone growth, which is driven by growth plate activity, in particular chondrocyte proliferation and hypertrophic chondrocyte expansion. The genetics of WMS and overlapping biochemical properties of ADAMTS10 and ADAMTS17 suggest that they interact in regulating bone growth[2]. Therefore, we hypothesize that ADAMTS10 and ADAMTS17 cooperate in an ill-defined molecular pathway that regulates growth plate activity.

METHODS: We generated *Adamts10* x *Adamts17* double knockout (DKO) mice by combining a published *Adamts10* knockout (KO) allele with an unpublished *Adamts17* KO allele[3]. We followed survival by Kaplan-Meier analysis and measured bone length after X-ray imaging. Growth plate architecture was analyzed by histomorphometry and immunostaining. Primary chondrocytes were isolated from neonatal rib cartilage and chondrogenesis examined by alizarin red staining. Primary mouse skin fibroblasts were isolated from WT, KO, and DKO mice and used to investigate the role of ADAMTS10 and ADAMTS17 in ECM deposition by immunostaining. For statistical analyses, we compared 2 independent samples with a Student's t-test and 3 or more samples with a one-way ANOVA followed by a posthoc Tukey test. A p-value of <0.05 was considered statistically significant. Mouse experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Icahn School of Medicine at Mount Sinai.

RESULTS SECTION: *Adamts10* x *Adamts17* DKO mice (n=7) showed significant lethality (Chi^2 <0.001) compared to individual KOs (n=11) or wild type (WT) mice (n=13), indicating that one *Adamts10* or *Adamts17* allele is required for postnatal survival (Fig. A, B). Bone length measurements after X-ray imaging of hind limb and forelimb long bones showed exacerbated bone shortening in the DKOs (n=3, p<0.05) compared to WT (n=8) or KO bones (n=8-9) suggesting a genetic interaction between *Adamts10* and *Adamts17* (Fig. C, D). This is further supported by significant intermediate bone shortening after deletion of one *Adamts17* allele in *Adamts10* KOs (n=9) compared to *Adamts10* KOs (n=9) or DKOs (n=3). Histologically, the proximal tibial DKO growth plates had significantly narrower hypertrophic zones while the proliferative zones were unchanged (n=3, p=0.036). Using immunostaining, we localized ADAMTS17 to the pericellular matrix of hypertrophic chondrocytes with increased signal intensity closer to the cartilage-bone interface. However, in-situ hybridization localized *Adamts17* mRNA to the perichondrium and primary ossification site in developing bones. These observations raise the possibility that *Adamts17* may be involved in the crosstalk between perichondrium and hypertrophic chondrocytes. To determine, if ADAMTS10 and ADAMTS17 are regulated during chondrocyte hypertrophy, we induced expression of hypertrophy-associated genes by culturing primary chondrocytes in the presence of okadaic acid and we quantified mRNA levels after 24h by real-time PCR. In addition to the hypertrophy marker *Col10a1*, gene expression of both, *Adamts10* and *Adamts17*, was significantly induced (n=3, p=0.009 and p=0.016, respectively), suggesting upregulation of *Adamts10* and *Adamts17* expression during chondrocyte hypertrophy. Finally, we used primary mouse skin fibroblasts to investigate ECM deposition of fibronectin and fibrillin-1, which represent key ECM scaffolds that are thought to be regulated by ADAMTS10 and/or ADAMTS17. Fibronectin fibers were absent in the ECM of *Adamts17* KO and *Adamts10* x *Adamts17* DKO fibroblasts (n=3, p<0.05). Intracellular fibrillin-1 accumulation was observed in *Adamts17* KO and DKO fibroblasts and in *Adamts10* KO fibroblasts in areas devoid of fibronectin. These data suggest a critical cooperative role of ADAMTS10 and ADAMTS17 in ECM scaffold formation, which may regulate growth plate activity.

DISCUSSION: Our data show that ADAMTS10 and ADAMTS17 cooperate in a biological pathway that regulates growth plate activity, bone growth, and ultimately human height. This is primarily supported by exacerbated shortening of the DKO bones and the fact that one allele of either *Adamts10* or *Adamts17* is required for postnatal survival. Mechanistically, we show that growth plate architecture is abnormal in the DKOs with a shorter hypertrophic zone. This is congruent with previous studies showing extended or attenuated hypertrophic zones in WMS *Adamts10* knock-in and *Adamts17* KO growth plates[4,5]. Our data suggest a dominant role for ADAMTS17 over ADAMTS10. Mechanistically, ADAMTS10 and ADAMTS17 could accomplish this through ECM regulation in the growth plate and/or at the cartilage-bone interface. In support of this hypothesis, we show defective ECM protein secretion and/or assembly in fibroblasts isolated from individual KOs and *Adamts10* x *Adamts17* DKOs. One open question is if ADAMTS10 and ADAMTS17 function as proteases, since they are both members of the ADAMTS protease family, or if they have non-canonical functions in the ECM. In support of the latter, ADAMTS10 is the only ADAMTS protease that cannot be activated by furin and ADAMTS17 undergoes autoproteolysis at multiple sites including in its catalytic domain, suggesting non-proteolytic functions in the ECM or at the cell surface[6,7]. Ongoing efforts focus on the characterization of chondrogenic differentiation and ECM secretion in WT, KO, and DKO primary rib chondrocytes and on resolving the question if ADAMTS10 and ADAMTS17 function as proteases or have non-canonical functions.

SIGNIFICANCE/CLINICAL RELEVANCE: Short stature syndromes, such as WMS, carry a large mental and physical health burden, especially in children, which can be the target of disrespect due to their short stature. A better understanding of the players involved in regulating human height, may provide new therapeutic targets to promote postnatal growth in affected children.



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