

ADAMTS6 is necessary for heart valve stratification and myocardium development

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Introduction: Congenital heart defects (CHDs) are the leading cause of child morbidity and mortality. ADAMTS6 is a protease known to regulate the extracellular matrix (ECM) and is necessary for heart development as patient variants lead to a myriad of CHDs. Additionally, *Adamts6*-deficient embryos have CHDs, including myxomatous heart valves and cardiomyopathy, and die at birth. However, the role of *Adamts6* in distinct cardiac lineages remains unknown.

Methods: *Adamts6* was conditionally inactivated in multiple cell lineages to isolate defects without the comorbidities witnessed in global *Adamts6* deletion. Since premature endocardial cushions become mature heart valves through epithelial to mesenchymal transition, *Adamts6* was conditionally inactivated in *Cdh5*Cre-positive endothelial cells to ascertain valve development. Additionally, *Adamts6* was conditionally inactivated in *Myh6*Cre-positive cardiomyocytes to ascertain its role in myocardium development.

Histology and immunofluorescence staining were performed to evaluate the morphology and composition of valves and muscle.

Results: *Adamts6^{fl/fl};Cdh5Cre* mice are viable, but showed significant pulmonary and aortic valve enlargement persisting into adulthood as compared to littermate controls. Histology revealed increased proteoglycan content and disorganized collagen (hallmarks of myxomatous valves). Immunofluorescence staining revealed an accumulation of fibrillin-1, a substrate of ADAMTS6. A decrease in collagen 1 and tropoelastin (components of ECM) immunofluorescence staining was also witnessed. Similarly, *Adamts6^{fl/fl};Myh6Cre* mice are viable, but showed a significant increase in trabecular myocardium and overall ventricular width.

Conclusion: These findings establish that ADAMTS6 plays a unique role in distinct cell lineages of the hearts and is thus essential for ECM organization and long-term integrity of heart function. Future studies will uncover specific substrates and molecular mechanisms in heart valves and myocardium via proteomics.