Genetic Defects in \textit{SHROOM3} Lead to Congenital Heart Defects

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Background and Hypothesis: Congenital heart disease (CHD) is the most common birth defect, but most genetic contributors remain unknown. We recently identified CHD patients with variants in a gene called \textit{SHROOM3}. The \textit{SHROOM3} protein impacts the actin cytoskeleton by binding ActinF and Rho-kinase, causing actomyosin constriction. \textit{SHROOM3} also binds Dishevelled2 (Dvl2), a component of Wnt/Planar cell polarity (PCP) signaling pathway, suggesting a connection between PCP signaling and actin-myosin contraction. We hypothesize \textit{SHROOM3} disruption alters PCP signaling and actin cytoskeleton during cardiac development, and is a novel contributor to CHD.

Project Methods: We analyzed the cardiac phenotype of \textit{Shroom3} gene trap knockout mice at embryonic day 14.5. We characterized the expression of \textit{Shroom3} during cardiac development using LacZ staining at important stages of cardiac development. Using IHC, we measured actomyosin disruption in \textit{Shroom3} knockout embryos. We preformed in silico analysis on previously identified \textit{SHROOM3} variants from patients with CHD.

Results: \textit{Shroom3} null mice had Ventricular Septal Defects (0.73, p=0.0006), Double Outlet Right Ventricle (0.33, p=0.04), Left Ventricle Noncompaction, and other CHD. \textit{Shroom3} mutant mice left ventricular wall thickness was 36\% thinner compared to wild type mice (99.0±8.6\textmu m, 63.0±8.4\textmu m, p=0.005). LacZ shows the expression of \textit{Shroom3} through important stages of cardiac development, and IHC shows actomyosin disruption. In silico analysis demonstrates CHD patients have \textit{SHROOM3} variants in highly conserved nucleic acid and protein sequences, and significant protein structural changes.

Conclusion and Potential Impact: \textit{Shroom3} null mice have cardiac defects resembling a Wnt/PCP disruption phenotype. Similarly, patients with CHD have likely pathogenic variants in \textit{SHROOM3}. These data support a role for \textit{SHROOM3} in CHD pathogenesis and begin to elucidate mechanisms. Identifying \textit{SHROOM3}'s role in CHD is critical to understanding cardiac development as well as the diagnosis, management and treatment of CHD.