# Genetic Defects in *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 *SHROOM3.* The SHROOM3 protein impacts the actin cytoskeleton by binding ActinF and Rho-kinase, causing actomyosin constriction. 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 *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 *Shroom3* gene trap knockout mice at embryonic day 14.5. We characterized the expression of *Shroom3* during cardiac development using LacZ staining at important stages of cardiac development. Using IHC, we measured actomyosin disruption in *Shroom3* knockout embryos. We preformed in silico analysis on previously identified *SHROOM3* variants from patients with CHD.

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

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