The Impact of *MYH6* Genotype on Hypoplastic Left Heart Syndrome

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Hypoplastic Left Heart Syndrome (HLHS) is a clinically and anatomically severe form of Congenital Heart Disease (CHD). Although prior studies suggest that HLHS has a complex genetic inheritance, its etiology remains largely unknown. The goal of this study was to characterize a risk gene for HLHS, both its role in etiology of HLHS and its impact on clinical outcome. We performed next generation sequencing on a multi-generational family with a high prevalence of CHD/HLHS, identifying a rare variant in the α-myosin heavy chain (*MYH6*) gene. A case-control association study of 190 unrelated HLHS subjects was then performed and compared to the 1000 Genomes Project. Damaging *MYH6* variants, including novel, mis-sense, in-frame deletion, premature stop, *de novo*, and compound heterozygous variants, were significantly enriched in HLHS cases ($p<1x10^{-5}$, observed in 10.5% or 20/190 of HLHS cases), demonstrating HLHS association. Clinical outcomes analysis showed reduced transplant-free survival in HLHS subjects with damaging *MYH6* variants ($p=6x10^{-3}$). Transcriptome and protein expression analyses with cardiac tissue revealed differential expression of cardiac contractility genes, notably upregulation of the β-myosin heavy chain (*MYH7*) gene in subjects with *MYH6* variants. We subsequently used patient-specific induced pluripotent stem cells (iPSCs) to model HLHS *in vitro*. Early stages of *in vitro* cardiomyogenesis in iPSCs derived from two unrelated HLHS families mimicked the increased expression of *MYH7* observed in *in vivo* ($p<0.01$) while revealing defective cardiomyogenic differentiation. We are working on gene editing in *MYH6*:R443P using CRISPR/Cas9 technology. Isogenic controls will support the specificity of the aberrant phenotype observed in HLHS iPSC derived cardiomyocytes. Rare, damaging variants in the *MYH6* gene are enriched in patients with HLHS, they are associated with altered expression of contractility genes, and are predictive of poor outcome. The etiology of *MYH6*-associated HLHS can be informed by studying iPSCs, and the functional assays reported suggest utility in using iPSCs to evaluate clinical interventions.