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Using patient-derived induced pluripotent stem cells to model left ventricular outflow obstruction defects

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Introduction
Left ventricular outflow tract obstruction (LVOTO) is a subtype of congenital heart disease affecting one or more structures on the left side of the heart – left ventricle, aortic valve and thoracic aorta. At its most severe, LVOTO defects manifest as hypoplastic left heart syndrome (HLHS), in which the left ventricle is underdeveloped, and the systemic circulation depends on the persistence of fetal circulatory physiology. LVOTO defects are at least partially genetic in origin, as they are found to cluster in families.

We have sequenced the exomes of a cohort of 49 LVOTO patients and their family members. We have identified a very rare gene variant that segregates with disease in two unrelated families with multiple affected members with LVOTO defects. This gene has been shown to be important for heart development in mice and zebrafish, but not yet in humans.

We are studying the impact of this gene variant and the gene itself in cardiomyocyte (CM) and endothelial cell (EC) development and function using patient-derived induced pluripotent stem cells (iPSCs). We hypothesize that the gene is important in cardiac development, affecting either CMs or ECs or both, as ECs have been shown to contribute to CM growth and maturation.

Methods
We obtained iPSCs from a HLHS patient with the rare gene variant and his unaffected mother, and control iPSCs from a healthy donor. We made knockout and heterozygous cell lines for the target gene from the control iPSCs by using CRISPR/Cas9. We have differentiated iPSCs to CMs and ECs, and studied their differentiation by immunohistochemistry and qPCR.

Results
Our results indicate that the patient cells are able to differentiate to both CMs and ECs, however, there are differences in cardiac gene expression patterns during differentiation. Currently, we are analyzing the knockout and heterozygous cell lines in comparison with the patient and control cell lines.

Conclusions
Our results indicate that disease modeling in patient-derived iPSCs is a promising method to evaluate the pathogenicity of candidate genes in congenital heart disease.