Reelin zebrafish mutants as a model for lymphoangiocrine-mediated cardiac repair following inflammatory injury

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Background/Objective: Myocardial injury has become of particular interest in pediatrics in the context of Multisystem Inflammatory Syndrome in Children (MIS-C). MIS-C is a rare complication associated with the contraction of SARS-CoV-2 that causes inflammation throughout the body. The exact cause of MIS-C is not yet understood. Cardiac lymphatic endothelial cells (LEC) contribute to cardiac repair following injury through the release of lymphoangiocrine signaling. Reelin, a structural protein highly expressed in LECs, is likely implicated in this process. Potential pathologic alleles of *REELIN* were uncovered in MIS-C patients during whole-exon sequencing. This study proposes the generation of a founder line (F0) of *reelin* zebrafish mutants as a model for MIS-C pathogenesis and lymphoangiocrine signaling following inflammation.

Methods: Four single-guide RNA (sgRNA) were developed to redundantly target exons of zebrafish *reelin* to create biallelic mutations, mimicking loss-of-function alleles identified in MIS-C patients. Cas9 endonuclease protein and sgRNA will be microinjected into 1-cell stage zebrafish embryos. To model inflammation, poly (I:C) will be injected to simulate viral dsRNA, and ventricular resection will simulate cardiac injury. Whole-mount confocal imaging will assess heart size, morphology, and cardiomyocyte proliferation or hypertrophy. Two reporter lines, fli1a and pdgfrb, marking endothelial cells and mural cells, respectively, will be used as a method of phenotypic analysis.

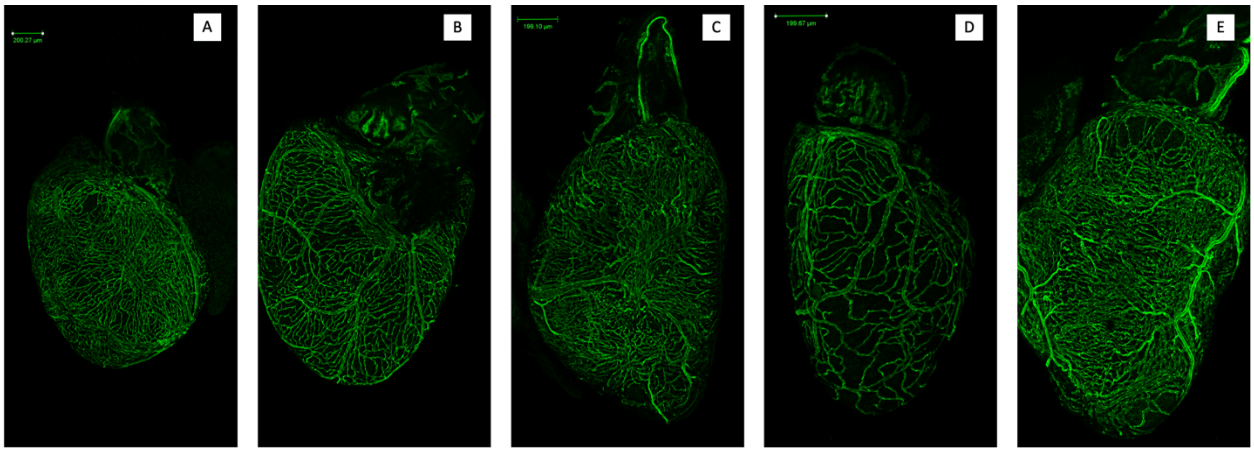
Results: Previous research on mouse knockout models for genes identified as regulators of cardiac lymphatic development has shown that LEC-mediated lymphoangiocrine signaling is important for cardiomyocyte growth during development. Reelin LEC-null mice, in general, grow smaller hearts with decreased cardiomyocyte proliferation and increased apoptosis. Extrapolating from research on mouse knockout models for genes identified as regulators of cardiac lymphatic development, we expect *reelin* mutants to develop myocardial phenotypes resulting from diminished cardiac lymphoangiocrine signaling. Inflammation models may exacerbate these phenotypes, resulting in worse cardiac function and lethality. Coronary artery vessel growth, seemingly related to coronary lymphatics, may similarly demonstrate dysregulated growth in *reelin* zebrafish mutants.

Conclusion: The expected results imply a novel role of Reelin as a mediator of lymphangiogenic signaling in zebrafish cardiac growth and pathological remodeling following inflammation. Validation of this model will facilitate screening of biomarkers for MIS-C pathogenesis, establishing the basis for preventative and therapeutic measures in susceptible pediatric populations.

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Figure 1. Example of exon 15 gRNA design targeting NM_005045.4(RELN):c.2932A>G (p.Thr978Ala) variant. Targets are specified by gRNA and are located 20 base pair upstream from NGG. Two oligos were used in a template-free PCR reaction to generate the gRNA DNA template, later to be transcribed and injected.

Figure 2. Confocal image analysis of WT (A), Apol1 gene mutant male (B, C), female (D) and



poly (I:C) injected fli1a:FGP reporter lines for enhanced green fluorescent protein (EGFP) in coronary vessels demonstrating assays to assess for cardiac phenotypes in reelin zebrafish mutants.

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