**Diagnosis and Molecular Characterization of Pediatric Myocarditis**

The incidence of Dilated Cardiomyopathy (DCM) is about 0.34 to 1.09 per 100,000 children per year [1]. In children, the small number of affected patients has challenged the development of effective age and disease-specific diagnostics and medical therapy.

It is estimated that approximately 30% of pediatric DCM is due to myocarditis. However, due to a lack of a rapid and non-invasive diagnostic test, the true incidence of myocarditis is difficult to determine. We have developed a simple method to identify circulating miRNAs as biomarkers of recovery in the pediatric DCM population [2, 3]. miRNAs are small RNAs that are stable in the circulation and can be used as biomarkers in several disease processes. We envision expanding this biomarker study to identify circulating miRNAs as biomarkers of diagnosis in pediatric myocarditis patients. To date the only study evaluating circulating miRNAs in pediatric myocarditis patients investigate expression of 3 miRNAs known to be associated with heart failure in adults but not in children [4]. Here we propose to utilize an unbiased approach that evaluates expression of 765 miRNAs in serum. We will compare circulating miRNA expression in pediatric myocarditis patients, normal controls (no heart disease) and pediatric heart failure DCM patients negative for myocarditis. These studies will be important to identify circulating miRNAs specific to myocarditis patients, and will aid in diagnoses of the disease.

In addition, we and others have shown profound molecular differences in pediatric and adult DCM [5-7]. However, molecular studies targeted to pediatric myocarditis patients are lacking. These studies would help identify novel targets for therapy in this patient population. We have previously shown that the transcriptome profile of pediatric DCM patients is unique and shares few similarities with adults [5, 6]. However, transcriptome changes in the pediatric myocarditis patients have not been investigated. The University of Colorado was recently awarded an NIH Trans-Omics for Precision Medicine (TOPmed) grant to RNA and DNA sequence over 800 adult hearts and 300 pediatric hearts, including pediatric hearts from transplant patients diagnosed with myocarditis. Although this will provide comprehensive genomics and transcriptomics data, the biological consequences of these changes are not always clear. To circumvent this problem, we initiated phenotypic characterizations of pediatric DCM hearts. These include characterization of lipid, metabolites and cellular composition. However, these initial characterizations did not include hearts from patients with myocarditis. In this application we propose to characterize hearts from pediatric myocarditis patients to identify novel targets of therapy.

Therefore, we propose the following Aims:

**Aim 1: Identify circulating miRNAs in myocarditis patients.** Circulating miRNAs will be identified using stored blood samples from myocarditis patients. Arrays containing 765 miRNAs will be used in these studies.

**Aim 2: In myocarditis patients perform metabolomics and lipidomics studies.** Metabolomics and lipidomics studies will be performed in explanted heart tissue from myocarditis patients. These results will be compared to non-failing controls and non-myocarditis DCM patients.

Completion of these studies will identify novel biomarkers to diagnose myocarditis in children and novel targets of therapy specific for this patient population.

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