Disease Pathogenesis and Progression in Congenital Myotonic Dystrophy

Congenital Myotonic Dystrophy (CDM) is a multi-systemic, dominantly inherited, disorder caused by a trinucleotide repeat expansion (CTG_n) in the DMPK gene. CDM occurs when the CTG_n hugely increases between the adult myotonic dystrophy type-1 (DM1) parent and the child. Children with CDM present at birth with respiratory insufficiency, talipes equiovarus, feeding difficulties and hypotonia. As children grow, they are at risk for intellectual impairment, autistic features, gastrointestinal symptoms, and motor delay. In adult DM1, many disease manifestations are due to sequestration of splicing proteins with the CTG_n repeat resulting in mis-splicing of many important proteins, a spliceopathy. However, it is not clear that a spliceopathy is responsible for symptoms during childhood, nor which splicing targets may be affected. Finally, in a tissue such as blood, which is not typically associated with symptoms, it is not clear whether change in splicing actually affects which RNAs are translated.

Currently, our group is conducting a study of disease progression in CDM, known as Health Endpoints and Longitudinal Progression in Congenital Myotonic Dystrophy (HELP-CDM). This study is designed to understand the clinical phenotype of CDM throughout childhood and identify important clinical outcomes. Such information will allow a standardized design of treatment trials in children to assess promising new therapies, such as anti-sense oligonucleotides (ASOs) or protein replacement approaches.

While the current study addresses the need to develop appropriate clinical endpoints, additional investigations are needed to identify the underlying pathological mechanisms that may cause disease manifestations. This proposal seeks to better understand the pathogenesis of CDM to achieve a better understanding of disease progression and prepare for approaching therapeutic trials. **We hypothesize that RNA splicing changes are responsible for disease progression in childhood. A secondary aim of the study is to identify blood-based biomarkers for therapeutic trials, either as RNA splice variation or other observed protein changes.** This study will utilize the HELP-CDM cohort which has enrolled sixty children with CDM and 30 control subjects between the ages of 0-13, and their affected parents in order to address the following ***Specific Aims***.

1. *To identify disease-causing RNA splicing changes in CDM patients.* RNA splicing ratios will be evaluated in peripheral blood mononuclear cells of patients and controls. Initial identification will seek to confirm the presence of RNA splicing changes previously identified in adult DM1 patients. Additional exploratory analysis will identify novel splicing changes specific to the CDM patients. We hypothesize that adult DM1 splicing changes may appear in late childhood and that novel splicing changes, including MTMR1 and BIN1, may be pathogenic early in childhood.
2. *To identify resulting change in translation in CDM patients and develop a tissue repository for studying disease pathogenesis.* From the blood samples, we will perform ribosomal profiling to identify whether the identified changes in RNA splicing effect which RNAs are translated. Some patients enrolled in this study will have muscle biopsies, which will permit confirmation of identified RNA changes within the muscle tissue. In addition, PBMC cells will be converted to lymphoblastoid cell lines to allow for future confirmatory experiments in MBNL knockdown to better understand the pathogenesis.
3. *To evaluate associations of identified RNA splicing changes and previously described changes in prostaglandin E2 and adiponectin with clinical endpoints obtained in the HELP-CDM study.* Measures of neuropsychiatric function, resting state functional MRI, IQ, oral and facial strength, muscle strength and function, cardiac conduction, respiratory function, and quality of life obtained annually for three years. Identified changes in RNA splicing in Aim 1 will be correlated with these clinical endpoints to connect the underlying pathophysiology with the clinical phenotype. Longitudinal study of both RNA splicing and clinical endpoints permits understanding of relative responsiveness and development of a potential biomarker. We hypothesize that distinct, age dependent, splicing changes may correlate and be predictive of the identified clinical endpoints.

Achieving these Aims will have immediate significance by providing essential information on genetic modifiers of anticipation and the pathophysiology of CDM through childhood. The results, when combined with the HELP-CDM study, will provide a complete clinical and biological model for CDM disease progression. This critical information will permit development of appropriate clinical and biological endpoints for therapeutic trials. Finally, completion of these Aims will provide the necessary experience in laboratory techniques to allow the applicant to become a leader in translational research in neuromuscular medicine.