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Standard Operating Procedures (SOPs) for Evaluating the Heart in Preclinical Studies of Duchenne Muscular Dystrophy

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Abstract

A recent working group meeting focused on Contemporary Cardiac Issues in Duchenne muscular dystrophy (DMD) was hosted by the National Heart, Lung, and Blood Institute in collaboration with Parent Project Muscular Dystrophy. An outcome of this meeting was to provide freely available detailed protocols for preclinical animal studies. The goal of these protocols is to improve the quality and reproducibility of cardiac preclinical studies aimed at developing new therapeutics for the prevention and treatment of DMD cardiomyopathy.

Reproducibility is a key component of research. This critical feature of research is especially true when investigating disease mechanisms and therapeutic interventions. Millions, or even billions of dollars and years of work could be spent to chase a "promising" experimental artifact [1]. The study of Duchenne muscular dystrophy (DMD) is no exception.

DMD is an X-linked recessive degenerative muscle disease and the most common childhood form of muscular dystrophy of childhood. DMD boys typically start to show muscle

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weakness between 2 and 5 years-of-age. Progressive muscle deterioration and fibrofatty replacement of muscle lead to the loss of ambulation during the teenage years and clinical signs and symptoms of cardiac involvement thereafter. Patients typically die prematurely due to cardiac or pulmonary complications, although with recent improvements in ventilatory support, cardiomyopathy is becoming an increasing cause of morbidity and mortality in DMD patients.

DMD is caused by mutations in the dystrophin gene. A complete absence of dystrophin results in DMD. Partial defects in the structure and/or quantity of dystrophin lead to the milder Becker muscular dystrophy (BMD) phenotype. Cardiac selective loss of dystrophin causes X-linked dilated cardiomyopathy (XLDC). Duchenne cardiomyopathy refers to heart-associated clinical manifestations seen in DMD, BMD and XLDC. Dilated cardiomyopathy is the characteristic clinical manifestation. Involvement of the conduction system can result in arrhythmia and sudden cardiac death. The current standard of cardiac clinical management is periodic heart function monitoring, timely administration of symptom-relieving and/or cardio-protective medications, and occasionally implantation of assist devices and transplantation. In DMD patients, the only current standard-of-care treatment is glucocorticoids (prednisone, prednisolone or deflazacort).

The current lack of disease-specific therapies signifies an urgent need to better define pathogenic mechanisms and to test drug/gene/cell therapeutics in animal models. Mammalian and non-mammalian animal models have been generated for dystrophin-deficient myopathies [2]. The cardiac phenotype in some of these models can be used for preclinical testing of novel Duchenne cardiomyopathy therapies such as exon-skipping antisense oligonucleotides, read-through drugs, adeno-associated virus-mediated micro-dystrophin gene therapy, and therapies targeting downstream pathogenesis. Unfortunately, a set of reference standard operating procedures (SOPs) dedicated for Duchenne cardiomyopathy studies was missing. This critical knowledge gap was identified as a major barrier according to a recent workshop (July 10–11, 2014; Bethesda, MD) organized by the National Heart, Lung, and Blood Institute (NHLBI), and sponsored by Parent Project Muscular Dystrophy (PPMD) [3]. To fill this gap, a task force committee was organized to develop and update SOPs for Duchenne cardiomyopathy animal studies by incorporating the state-of-the-art cardiac assessment and monitoring methods and procedures.

The "Cardiac Protocols for Duchenne Animal Models" were developed by the following method. The authors (and members of the animal model task force) first generated a list of protocols thought to be crucial to improve reproducibility between different cardiac studies. Each SOP topic was assigned to 2–3 task force members. The leads for several of the SOPs collected protocols from their own, and other members' labs and wrote detailed protocols that combined critical components of each. In some cases alternative steps with advantages for different applications were included. The authors for each SOP then reviewed the protocols, which were then further reviewed by the task force chairs prior to posting.

The resulting SOPs are posted on the PPMD website (http://www.parentprojectmd.org/site/PageServer?pagename=Advance_researchers_sops). These freely available SOPs complement the existing TREAT-NMD protocol repository (http://www.treat-nmd.eu/

resources/research-resources/dmd-sops/). Each SOP has six sections including: objective, cautions, materials, methods, evaluation and interpretation of results, and references. These protocols are designed to serve as a reference point among laboratories and should not be construed as mandatory. As “living documents”, we expect to annotate the existing protocols with comments and improvements based on user feedback. We further expect to expand the SOP repository by incorporating new methodologies as the field moves forward.

Lack of reproducibility has become an increasingly serious concern in research involving animals in recent years [4, 5]. Among many factors that have contributed to this problem are insufficient methodologies and procedure errors in the execution of experiments. We expect the publication and free access of the PPMD cardiac protocols for DMD animal models should help to improve the standardization and comparability among different laboratories and minimize protocol-related issues in preclinical studies.

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