Non-invasive characterisation of early disease progression in mouse models of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a muscle wasting disease that typically affects young boys. Characterised by its early onset and rapid progression, boys affected by the DMD lose ambulation rapidly and are typically wheel-chair bound before their teenage years. Natural progression studies of affected boys have done much to map disease manifestation. In a clinical setting patients are assessed for a number of criteria including body composition, both fine and gross measures of mobility and strength, respiratory and cardiac function. By being able to quantify disease progression and therefore its prevention, researchers are able to more effectively gauge treatment efficacy. Before therapeutics reach patients judicious pre-clinical testing is paramount and requires animal models that phenotypically parallel DMD disease progression.

A number of mouse strains have been developed to model the debilitating disease; the gold standard of these is the *mdx* (dystrophin null) mouse which has a mild pathology. The dystrophin and utrophin (utrn) double knockout (*dko*) is more severely affected and develops a pathology that more closely resembles DMD progression in patients. Our aim was to develop and characterize a non-invasive battery of tests capable of ascertaining disease progression that can be later exploited for therapeutics development. Previous studies have explored isolated time-points, employed mouse models with limited clinical applicability or used assays predisposed to user bias or mouse temperament. To overcome these limitations, four strains of mice (wild-type, *mdx*, *mdx*:utrn$^{+/-}$ (*het*) and *mdx*:utrn$^{-/-}$ (*dko*)) were studied from 4-16 weeks of age. This period corresponds with early stage DMD when clinical interventions are likely to be of most benefit. The temporal profile of body composition (using nuclear magnetic resonance), presence of cardiac arrhythmia (via electrocardiogram) and gait abnormalities (via the DigiGait^{TM} treadmill gait analysis system) were assessed, with the hypothesis that these factors would segregate with disease progression and be useful indicators to monitor during pre-clinical therapeutics testing. All experiments were conducted in accordance with the code of practise for the care and use of animals for scientific purposes, as stipulated by the NHMRC.

This study identified that *dko* mice did not develop cardiac arrhythmia between 4-16 weeks of age. Analysis of body composition identified inherently low levels of lean and fat mass in the *dko* and the rate of lean and fat mass accretion was significantly reduced in the severely affected mouse compared to other strains. Regarding gait, the *dko* mice displayed significant hindlimb impairment that worsened from 4-9 weeks, paralleling human disease progression. *Dko* mice had reduced left hindlimb stance duration (when paws are in contact with the treadmill belt) and therefore spent a longer time in swing (movement) over time, indicative of poor left hindlimb control. Compared to wild-type and mildly affected mice, the hindlimbs of *dko* mice were turned inward towards the midline, as indicated by reduced hindlimb stance width and reduced paw angles. *Dko* mice had consistently reduced stance width variability in both hind- and forepaws, indicative of constrained limb movement. A critical finding of these experiments was that mice could be stratified into two groups; mice that reached the experimental endpoint at 16 weeks of age and those that did not, due to death or having scored above a pre-determined criterion end-point and therefore requiring early termination. It was then possible to assess factors that segregated in a similar fashion. Surprisingly, fat and lean mass were similar between the two groups. Remarkably, DigiGait analysis revealed that the capacity of mice to walk at a speed of 15 cms^{-1} at 8-9 weeks is a powerful predictor of survival at 16 weeks. The absence of cardiac arrhythmia was a surprising finding given it is a major contributor to DMD fatalities. In conclusion, body composition, gait abnormalities and speed maintenance but not cardiac arrhythmia, delineates disease progression within the first 16 weeks of post-natal development, providing a method of non-invasively assessing the treated DMD models in a pre-clinical setting.