Biomarker Discovery in Duchenne Muscular Dystrophy Through Global Metabolomic Analysis

Lee-McMullen, B.; Chrzanowski, S.; Vohra, R. (UF, Physiology); Edison, A.S. (UF, Biochemistry); Vandenborne, K. (UF Physical Therapy); Walter, G. (UF, Physiology)

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that results from mutations in the dystrophin gene and is characterized by progressive muscle deterioration, loss of functional abilities and reduced life expectancy[1]. Due to the lack of a curative therapy, several clinical trials are in progress; however, outcome measures of DMD trials often have several inherent limitations[2]. As a result, there is a pressing need for a biomarker capable for tracking therapeutic efficacy in these clinical trials for DMD. In this study, we used a preclinical model of DMD, the *mdx* mouse, and a minimally invasive global metabolomics investigation of serum using nuclear magnetic resonance (NMR) for biomarker discovery tracking disease progression in DMD.

Experimental

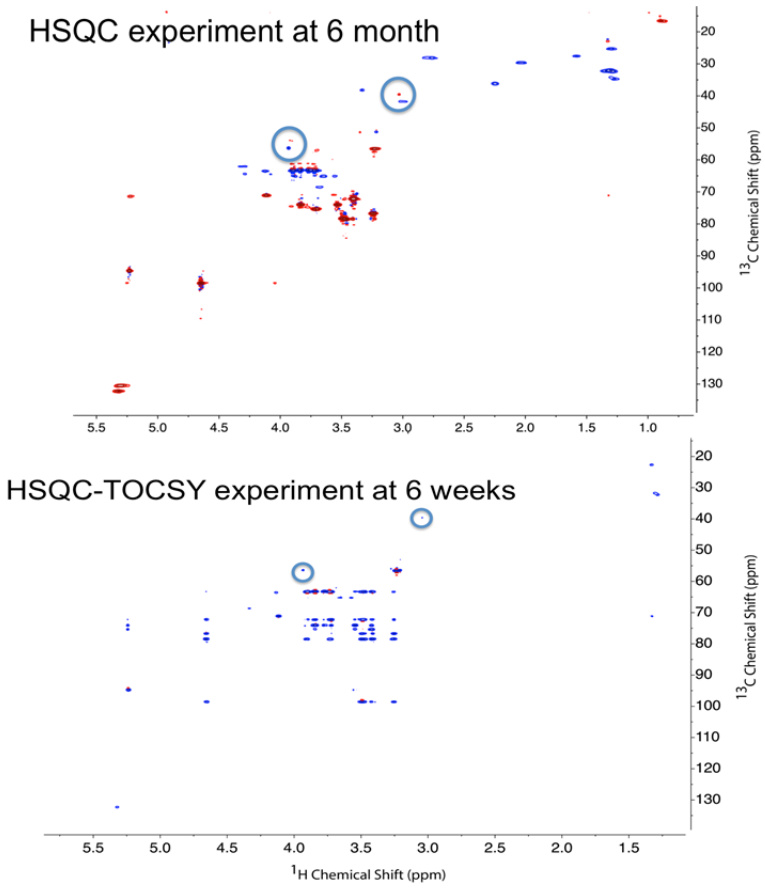
Serum samples were obtained through submandibular puncture in *mdx* mice at either 6 weeks or 6 months of age. Following clotting and centrifugation of whole blood samples, serum were collected and analyzed with one-dimensional (1D) and two-dimensional (2D) spectroscopy acquisitions. Both ^{1}H and ^{13}C nuclei were investigated using an Agilent 600 MHz high-resolution NMR equipped with a custom HTS probe[3], which has been shown to have two times more mass sensitivity than commercially available probes. Two-dimensional experiments were heteronuclear single quantum correlations (HSQC) between ^{1}H and ^{13}C, which reveals protons that are directly bonded with a carbon. The 6 weeks old spectra included a total correlation spectroscopy within the HSQC pulse sequence (HSQC-TOCSY) adding if the protons bonded to the carbons are within the same spin system

Results and Discussion

Previous analysis of 6 months serum revealed that peaks indicative of creatine at 3.02 ppm in ^{1}H spectra did not show the corresponding peaks in the carbon spectra at 39 ppm. On further analysis of the 2D spectra at 6 months, we found that there are multiple resonances in a similar area making it difficult for a positive identification (Figure 1a). In the 6 week spectra, we found that there was a single resonance agreeing with that predicted for creatine (Figure 1b).

a.

b.



Conclusions

In analysis of serum from both 6 month and 6 week old *mdx* mice, we find that there may be more than a single metabolite contributing to the resonances typically indicative of creatine. Further NMR and MS analysis is being performed to identify these unknown resonances and their association with disease progression.

Acknowledgements

We would like to acknowledge our funding sources including National High Magnetic Field Laboratory, the TL1 training grant from the CTSI, the NMPT T32 Fellowship and NIH grants (SECIM; U24DK097209, U54AR052646).

References

[1] Hoffman E.P., *et al.*, CELL, **51**, 919-928 (1987).
[2] Finkel R.S., *et al.*, PLoS ONE, **8(12)**, (2013).
[3] Ramaswamy, V. *JMR* **2013**.

Figure 1. (a) 2D HSQC experiment on serum from 6 months old *mdx* mouse. (b) 2D HSQC-TOCSY experiment on serum from a 6 week old *mdx* mouse.