Metabolomic Analysis of Urine to Track Disease Progression in Duchenne Muscular Dystrophy

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Introduction

Duchenne Muscular Dystrophy (DMD) is an X-linked genetic disorder in which the absence of dystrophin protein causes sarcolemma fragility resulting in incorrect muscle cell physiology and function[1]. Eventually failed muscle regeneration leads to the progressive loss of ambulation in early teens, and the boys typically succumb to the disease in third decade of life. Despite the rapid rate of disease progression, there are few accepted surrogate endpoints and the FDA approved clinical outcome measure lacks sensitivity[2]. As a result, there is a dire need for a biomarker to track disease progression in DMD. Urine is a non-invasive biofluid that is easily acquired from all ages. In this study, urine samples from boys with and without DMD are analyzed using global NMR based metabolomics to investigate metabolites that track disease progression

Experimental

In conjunction with an ongoing natural history study, ImagingDMD (imagingdmd.org), we are collecting urine from consenting boys with DMD and controls from a cohort of 125 boys. This gives us the unique opportunity to also have additional metadata including their detailed subject phenotype, rate of disease progression and other clinical measures. As a preliminary investigation of the data from the cohort we ran 25 urine samples (n=21 DMD, n=4 control) using a 600 MHz Bruker Avance II NMR. Samples were prepared in an NMR tube with 200 uL of a sodium phosphate buffer and 400 uL of urine and 1mM DSS as an internal standard. One-dimensional (1D) noesypr were acquired and analyzed.

Results and Discussion

These preliminary results suggest that there is a significant difference between DMD and control urine from boys 5-15 years old. Using Partial Least Squares Discriminant Analysis (PLS-DA), we found that we could differentiate between DMD and control urine based on the amount of creatine and creatinine (data not shown). In Figure 1 we show that in DMD urine there is a significantly greater amount of creatine than what was observed in control urine. Conversely, we found that there is a depletion of creatinine in the DMD urine as compared to the controls. We did not find differences in urine succinate levels. Using this preliminary metabolomic data we have found a modest but significant correlation MRI determined biomarkers of disease progression (Muscle proton relaxation times) and the creatine/creatinine ratio. (data not shown).

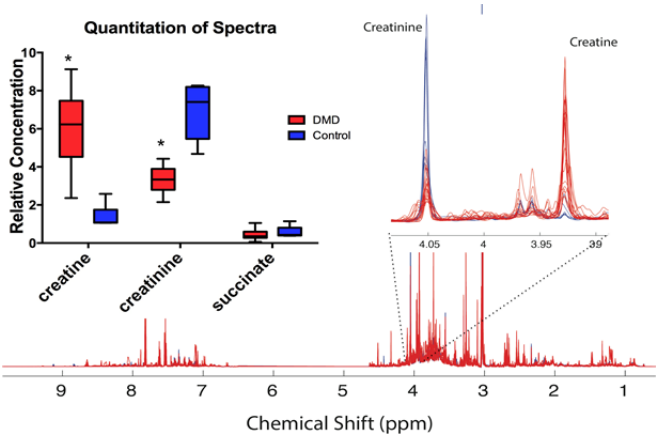

Figure 1.
We analyzed 25 human urine samples from boys with muscular dystrophy and age-matched control boys.

Conclusions

We used global NMR metabolomics to identify and quantitate metabolites found in the urine of boys with DMD in comparison to controls. Overall, we found that we were able to use both urine levels of creatine and creatinine to identify DMD urine and possibly to track disease progression in the future.

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References

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[2] Finkel R.S., *et al*., PLoS ONE, **8(12)**, (2013).