A Metabolomic Analysis of Very-Long Chain Acyl-CoA Dehydrogenase Deficient and Corrected Mice

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Introduction

Very long-chain acyl-CoA dehydrogenase deficiency is a condition that prevents the body from metabolizing certain very long-chain fatty acids (>22 carbons). The break down of these fatty acids occurs through the enzyme very long-chain acyl-CoA dehydrogenase (VLCAD). When this enzyme is lacking the regular process of shuttling in fats into the mitochondria to be metabolized is stopped. Carnitine moves macromolecules into the mitochondria, but when they are not metabolized there is an accumulation of fatty acids intermediates resulting in a decrease in the glucose production. People with this deficiency tend to be lethargic and hypoglycemic. Currently, many studies are looking to diagnose early in age[1] and treat using gene therapies[2]. In this project, we analyzed the serum of mice with and without the VLCAD enzyme and one VLCAD deficient group that was corrected through the viral expression of the VLCAD enzyme using an adeno-associate virus (AAV). We hypothesize that there is a metabolic signature that will be sensitive to VLCAD expression to track gene correction non-invasively.

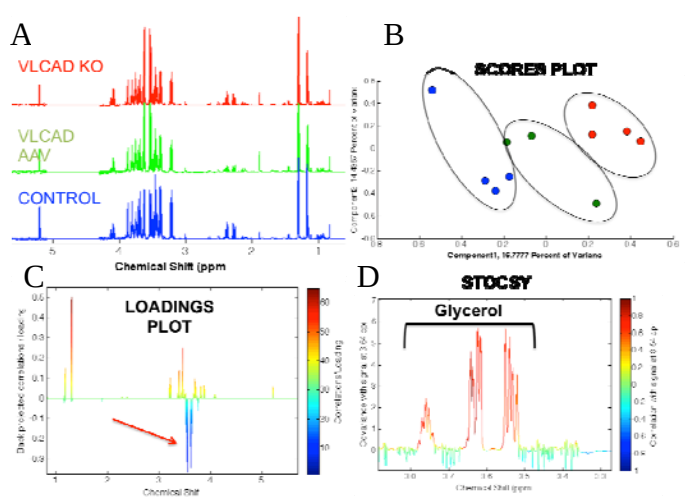

Figure 1. A) Overlay of spectra from the three groups. B) Scores plot showing separation of the groups using PLS-DA. C) Loadings plot showing that peak 3.64 ppm is important in separating the groups. D) Peaks that co-vary with peak 3.64 reveal a spectrum of glycerol.

Experimental

We used serum from 4 VLCAD knock out (KO) mice, 4 VLCAD KO mice treated gene therapy (AAV) and 4 control mice. Whole blood was processed and the serum was stored at -80°C. The serum was then thawed and 100ul of plasma and 50ul of 0.9% NaCl solution with 10% D_2O was added to a 2.5 mm NMR tube[3]. Samples were analyzed at 25 degrees Celsius on the 600 MHz Avance II magnet with a 5 mm cryoprobe. Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences were used to filter out broad signals from residual proteins in the sera. Serum spectra were processed using the NMRPipe, including phasing, zero filling and baseline correction. Processed spectra were then analyzed using an in-house Metabolomics Toolbox in Matlab, including normalization and statistical tests including principle component analysis and statistical correlation spectroscopy (STOCSY)[4].

Results and Discussion

Preliminary multivariate analysis of the mouse serum shows differences between the three groups. The loadings plot (Figure 1C) displays the peaks that we found were important in separating the groups of mice. Based on peaks that have similar variances as these peaks we attributed the major differences are due to glycerol.

Conclusions

This analysis reveals the metabolic intermediate glycerol as being enhanced in the serum of mice without VLCAD. Moreover, when VLCAD levels are increased following viral delivery using AAV there is an apparent renormalization of glycerol. In addition, VLCAD deficient mice were found to have higher levels of serum sugars compared to both treated VLCAD KO and control mice. These changes in NMR detectable serum metabolites are in agreement with the pathophysiology of the disease previously observed[5]. These early data indicates that ^{1}H metabolomics measurements of serum may be a minimally invasive means to track VLCAD activity in vivo.

Acknowledgements

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References: [1] Ficicioglu C et al, I Pediatr 2010 [2] Merritt et al. Mol Therapy 2009 [3] Beckonert et al. Nature Prot. 2007 [4] Cloarec et al. Anal Chem 2005 [5] Keeler et al, Mol Therapy 2012