CHILDREN'S MEDICAL CENTER RESEARCH INSTITUTE AT UT SOUTHWESTERN

Howard Hughes Medical Institute

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Large Scale Profiling of Fibroblasts from Pediatric Patients with Inborn Errors of Metabolism Results in the Identification of Siblings with L-2-Hydroxyglutaric Aciduria

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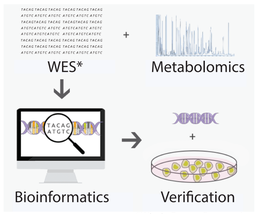
Mass Spectrometry: Lauren Zacharias, MS, Danny Vu, PhD, Feng Cai, PhD, Tom Matthews, PhD, Misty Martin Patient Samples: Chunxiao Pan, PhD

The Genetic and Metabolic Disease Program Workflow

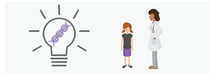
1. New Disease Suspected

2. Metabolic and Genomic Profiling



*Whole Exome Sequencing

3. Identification of Causative Genetic Mutation

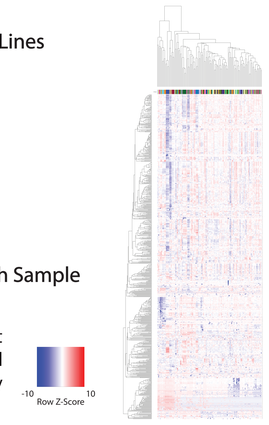


4. Development of Novel Therapeutics

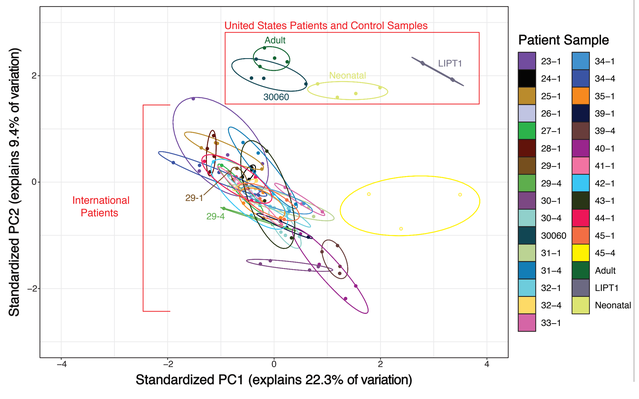
Experimental Design: Metabolomics

- 29 Patient-Derived Fibroblast Cell Lines
- 1 Neonatal Fibroblast Cell Line
- 1 Adult Fibroblast Cell Line
- 123 Samples Total (4 replicates in most cases)
- 681 Metabolites Evaluated for Each Sample

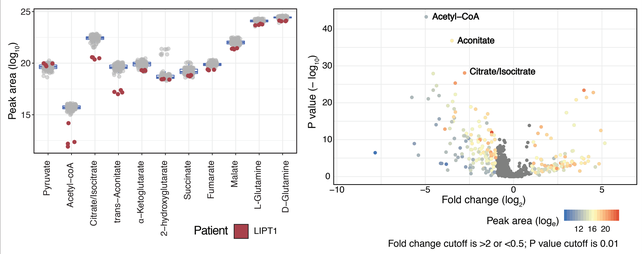
Here, we created a heatmap that demonstrates clustering of biological replicates and metabolic heterogeneity between patient samples.



Principal Component Analysis Plot



Confirmation of Metabolic Changes in Lipoyltransferase-1 Deficient Cells



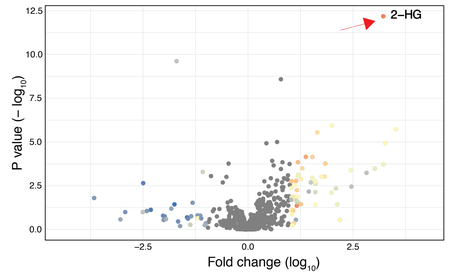
Broad metabolic changes predominately in TCA cycle metabolites are evident in the LIPT1 patient-derived cells.

Diagnostic Work-up for Siblings: Patients 29-1 and 29-4

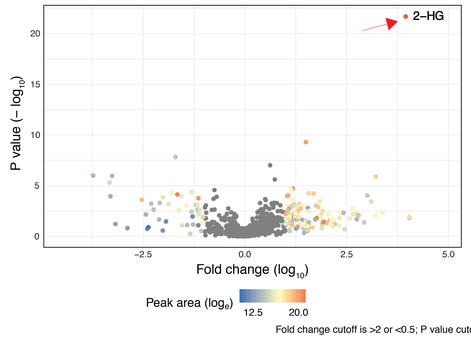
- 15 year old (patient 29-1)
- 18 year old (patient 29-4)
- Female
- Intellectual Impairment
- Ataxia
- Elevated Excretion of Glutaric Acid
- Urine Organic Acids

Differential Analysis of Metabolites in the Siblings' Cells Reveals Elevated 2-HG

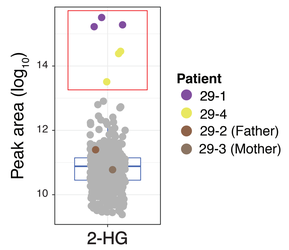
Patient 29-1 (~12-fold increase)



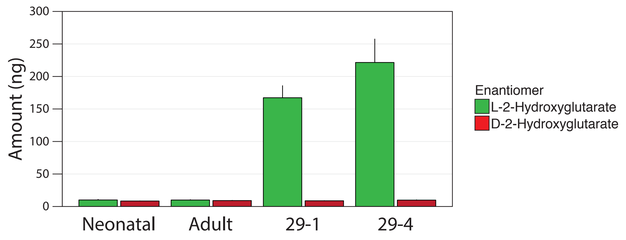
Patient 29-4 (>20-fold increase)



Normal Plasma 2-HG Levels in the Parents of Patients 29-1 and 29-4

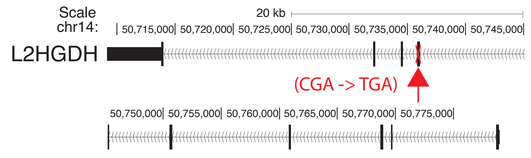


The L-2-HG Enantiomer is Exclusively Elevated in Patients 29-1 and 29-4



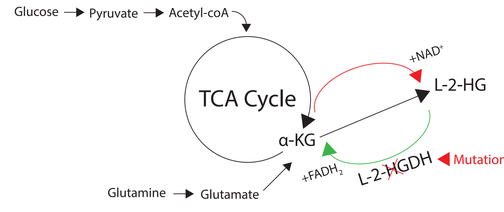
The bar graph shows nanograms per two million cells of the L-2-HG and D-2-HG enantiomers. Derivatization-based mass spectrometry methods indicate that L-2-HG alone exceeds physiological concentrations.

Whole Exome Sequencing Reveals L-2-HG Dehydrogenase Mutation



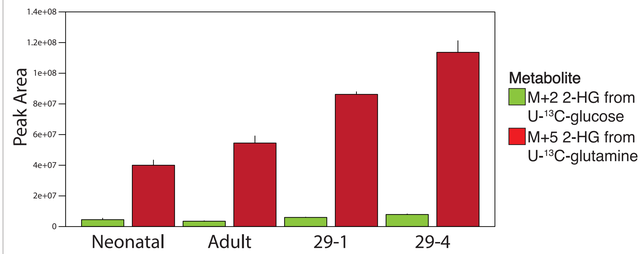
Patients 29-1 and 29-4 are homozygous for the above mutation that introduces a stop codon into the L-2-hydroxyglutarate dehydrogenase gene. The parents are heterozygous for this mutation. Image from: Human Feb. 2009 (GRCh37/hg19) Assembly; http://genome.ucsc.edu

L-2-HG Dehydrogenase Function



Information and aesthetic drawn from: (Kranendijk et al., 2012; Mullen et al., 2011)

2-HG is Predominately Derived from Glutamine rather than Glucose



Here, ^{13}C-labeled glucose and ^{13}C-labeled glutamine were introduced to fibroblasts derived from patients 29-1 and 29-4. Normalized peaks indicate the amount of 2-HG derived from glucose (green) and glutamine (red).

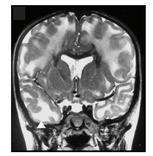
Conclusion

We designed a metabolomics platform that uses large-scale, unbiased methods to better identify meaningful metabolic outliers. Our system, which captures metabolic differences in fibroblasts derived from patients with inborn errors of metabolism, has the potential to enable more efficient diagnosis of these children. Here, L-2-hydroxyglutaric aciduria was identified as the inborn error of metabolism afflicting two siblings. Further experimentation, namely metabolic flux analysis, offered mechanistic insight into the disease, indicating that L-2-hydroxyglutarate is predominately made from glutamine rather than glucose.

Future Directions

Objective: Gain an understanding of the mechanism by which elevated L-2-HG hinders brain development and investigate the role of altered glutamine utilization

Background: Leukoencephalopathy is a hallmark of the disease. Initial diagnosis of L-2-HG aciduria is usually made through observation of subcortical white matter abnormalities and cerebral atrophy on MRI (Fourati et al., 2016; Kranendijk et al., 2012; Ma et al., 2017) .



Coronal T2 FSE Weighted Showing White Matter Anomalies. Reprinted from "MRI features in 17 patients with l2 hydroxyglutaric aciduria" by Fourati et al., 2016, Eur J Radiol Open, 3, p. 245-250. Copyright 2016 Elsevier Inc.

Aim: Use model systems, namely patient-derived fibroblasts reprogrammed into induced pluripotent stem cells and brain organoids, to study abnormal central nervous system development from a novel perspective

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