MULTI-OMICS STUDIES IN PATIENT-DERIVED AND CRISPR-EDITED CELLULAR MODELS OF METHYLMALONIC ACIDEMIA AND PROPIONIC ACIDEMIA REVEAL DYSREGULATION OF SERINE METABOLISM: NEW DIRECTIONS FOR CELLULAR PATHOGENESIS IN DISORDERS OF BRANCH CHAIN AMINO ACID METABOLISM

Arianna Anzmann[1], Veronica Busa[1], Susan McRitchie[2], Susan Jenkins Sumner[2], Hilary Vernon[1]

[1]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD , [2]Nutrition Research Institute, University of North Carolina, Kannapolis, NC

Here we show disturbances in serine transport, de novo synthesis and downstream utilization in two closely related disorders of branch chain amino acid metabolism: methylmalonic acidemia and propionic acidemia. Serine biosynthetic pathways are of particular interest because they have recently been implicated in the pathogenesis of multiple mitochondrial disorders, and have the potential for therapeutic targeting. Via untargeted proteomics analysis in fibroblasts derived from patients with methylmalonic acidemia (MMA) and propionic acidemia (PA) we identified upregulation of multiple proteins involved in serine synthesis and thiol metabolism, including phosphoserine amino transferase (PSAT1), cystathionine beta synthase (CBS) and mercaptopyruvate sulfurtransferase (MPST). Transcription factor analysis of genes encoding for the differentially expressed proteins provides evidence for induction of ATF4 mediated stress responses, which regulate the expression of genes involved in mitigating oxidative stress and promoting amino acid synthesis. Next, via untargeted metabolomics analysis in plasma from affected individuals, we identified significantly increased excretion of cystathionine and glutathione, central metabolites in serine and thiol metabolism. In order to provide further mechanistic insight into these observations, we developed CRISPR edited *MUT*null and *PCCA*null HEK293 cells. These cells closely recapitulate the primary defects of MMA and PA including significant increases in excretion of propionylcarnitine (*MUT*nulland *PCCA*null cells) and in methylmalonic acid (*MUT*null cells). We show that expression of key genes associated with serine and thiol metabolism are upregulated in these cells, including *CBS* and *PSAT1*, as well as increased expression with other canonical genes associated with the ATF4 stress response. Finally, we employed flux metabolomics analysis to trace utilization of labeled glucose in CRISPR edited *MUT*null and *PCCA*null HEK293 cells, and identified differences in serine transport, serine synthesis and downstream utilization. Together, these findings emphasize the overlapping pathology between organic acidemias and primary mitochondrial diseases, converging on alterations in serine and thiol metabolism, with potential for novel therapeutic targeting.