Biochemical diagnosis of fumarate hydratase (FH) deficiency disease

Monica Madya*[1,2], Alan Cahill[2], Kaitlyn Bloom[3], Amy Goldstein[4], Luis Fernandez[5], Steven Dobrowolski[6], Jerry Vockley[3,5], Rebecca Ganetzky[2,4,8]

[1]COL 2025, University of Pennsylvania; [2]Center for Computational & Genomic Medicine, Children's Hospital of Philadelphia; [3]Division of Genetic & Genomic Medicine, Children's Hospital of Pittsburgh; [4]Mitochondrial Medicine Frontier Program, Division of Human Genetics, Children's Hospital of Philadelphia; [5]Department of Pediatrics, University of Pittsburgh School of Medicine, [6]Department of Pathology, University of Pittsburgh School of Medicine; [8]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania , * mmadya@sas.upenn.edu (presenter's email)

INTRODUCTION

Oxidation of metabolites in the tricarboxylic (TCA) acid cycle produces reducing equivalents for mitochondrial oxidative phosphorylation (OXPHOS). Pathogenic variants in genes encoding for the essential enzymes and metabolites cause rare, often life-threatening diseases with multi-organ manifestations.

Fumarate hydratase (fumarase; FH) deficiency is an autosomal recessive disorder resulting in severe infantile-onset disease, severely affecting brain function and development [1]. This is likely due to impaired energy production and accumulation of fumarate, a highly reactive intermediate.

OBJECTIVES

Barriers to accurate, timely diagnosis of FH disease include disease rarity and a phenotype that overlaps other metabolic diseases. Biochemical diagnosis of FH currently relies on elevated fumarate levels in urine. However, atypical cases with pathogenic variants in *FH* have shown intermittently normal fumarate levels.

We investigated the case of an 18 month old boy with biallelic variants of uncertain significance in *FH* (p.L14Pf*42 and p.Q273R, confirmed in *trans*) who presented with neonatal hypothermia, infantile epilepsy, developmental regression, neutropenia and congenital brain anomalies; however, urine fumarate was normal. A more severe case of FH disease was also investigated. Cellular biochemical analyses were performed to characterize FH function. Cell lines are referred to as FH1 (attenuated phenotype) and FH2 (typical, severe phenotype).

METHODS

Protein expression of FH in fibroblasts was determined using Western blot. FH enzyme activity was analyzed using a colorimetric assay. Mitochondrial energetics was also measured using Oroboros oxygraph polarography on permeabilized cells in response to sequential TCA cycle substrates – fumarate, succinate, and malate – and ADP. Fumarate-driven oxygen consumption and complexes I and II rates were then calculated. Succinate dehydrogenase (SDH) activity was analyzed using a colorimetric assay with exogenous doses of fumarate. At least 3 replicates were performed for all assays.

FH ENZYME & MITOCHONDRIAL ENERGETICS

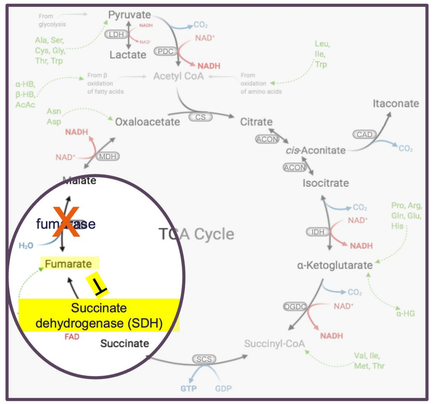


Figure 1. TCA cycle metabolites and hypothesized inhibitory effects of fumarate. Figure adapted from Bevital. [2]

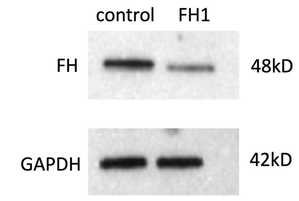
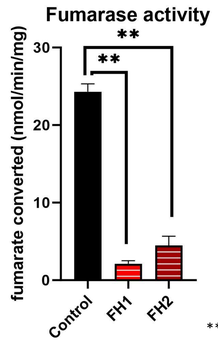


Figure 2. FH protein expression in control and FH1 whole fibroblasts, normalized as % to GAPDH. Band intensity was 71.8 for control and 29.2 for FH1.



**p<0.01

Figure 3. Comparison of fumarate-driven NADH production in control and FH1 fibroblasts.

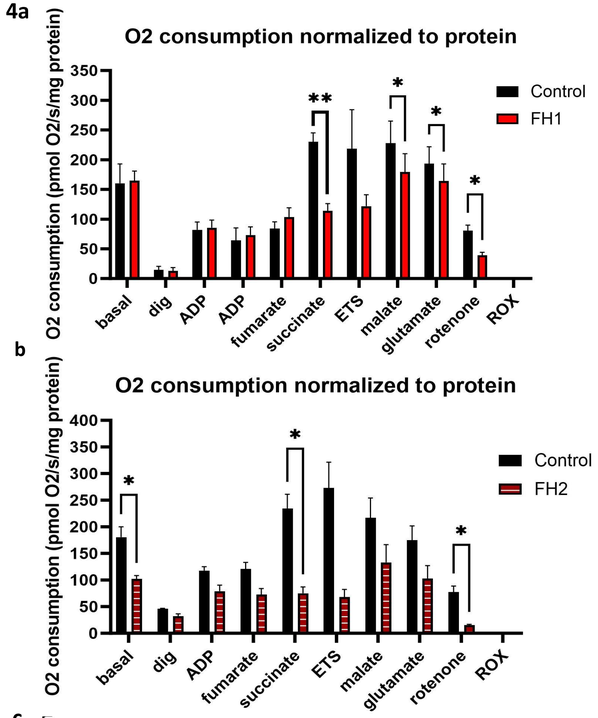


Figure 4. Oroboros respirometry in permeabilized fibroblasts after addition of sequential TCA cycle substrates to **a.** control vs FH1 **b.** control vs FH2 **c.** Complexes I and II rates of oxygen consumption. **p<0.001

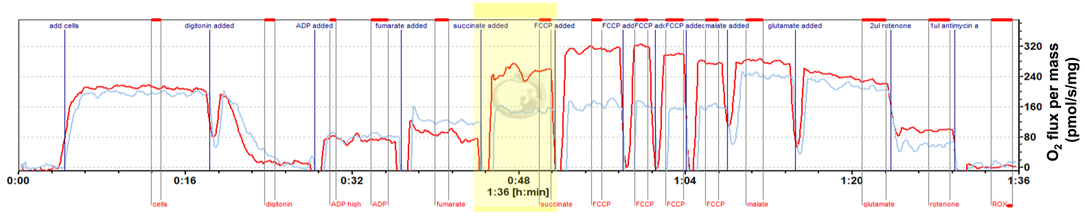


Figure 5. Time course of Oroboros oxygen flux per mg protein of control (red) vs FH1 (light blue)

SUCCINATE DEHYDROGENASE (SDH) ACTIVITY

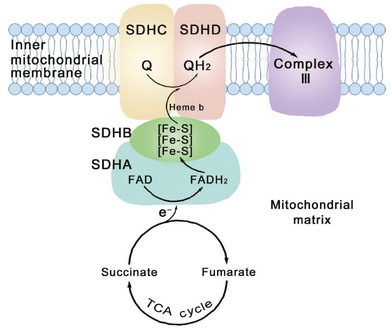


Figure 6. SDH as part of both the TCA cycle and electron transport chain. Figure adapted from Moosavi et al. [3]

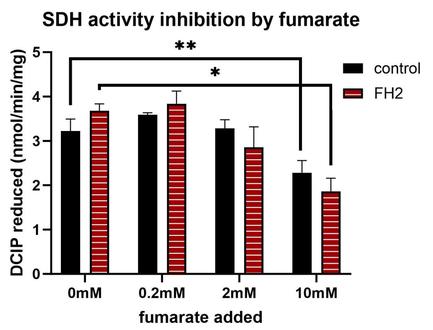


Figure 7. Increasing the concentration of fumarate causes dose-dependent inhibition of succinate dehydrogenase

CONCLUSIONS

The results in total confirm FH deficiency in the patient cell lines. This demonstrates that urine fumarate and fumarate:succinate levels can be normal despite classic levels of enzyme deficiency. Therefore, a higher index of suspicion is necessary to make an FH diagnosis.

FH deficiency is also shown to alter OXPHOS, causing both lower succinate (Complex II)-driven and uncoupled respiration (figure 4). Fumarate accumulation, characteristic of FH disease, likely causes the dampened Complex II activity (figure 7). The Oroboros respirometer could serve as a promising diagnostic tool to detect this respiratory impairment is currently only clinically available at the Children's Hospital of Philadelphia. The pathophysiology of FH disease is thus complex, and a multifaceted approach is needed for a conclusive FH diagnosis.

ACKNOWLEDGEMENTS

We would like to thank the families who provided samples for this study. Thank you also to Sheila Clever, Kristen Navarro, Dasi Long, Christine Kong, Tyler Coyne, Alyssa Tang, and the Center for Computational Genomic Medicine. This work was supported by the Pincus-Magaziner Family College Alumni Society Grant (Center for Undergraduate Research & Fellowships).

REFERENCES

[1] Ezgu, F., Krejci, P., & Wilcox, W. R. (2013). Mild clinical presentation and prolonged survival of a patient with fumarase deficiency due to the combination of a known and a novel mutation in FH gene. *Gene*, *524*(2), 403–406.

[2] Mosavi, B. et al. (2020). Genetic, epigenetic and biochemical regulation of succinate dehydrogenase function. *Biological Chemistry*, *401*(3), 319-330.

[3]Bevital. (2023, May 16). TCA Cycle Metabolites. Retrieved from https://bevital.no/tca-cycle-metabolites/ (2024, April 12)