The Feasibility of Improving Positive Predictive Values of MCAD Deficiency Screening Through the Use of Additional Acyl-Carnitine Markers

Jacob Voudren | Dr. Mark A. Morrissey

NEW YORK STATE | Department of Health | Wadsworth Center

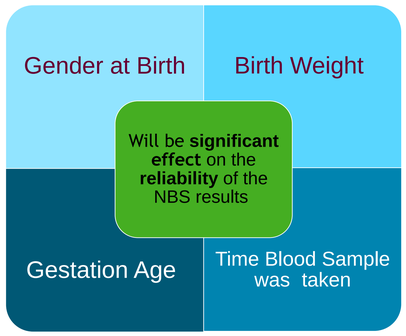
Abstract

Fatty-Acid Oxidation disorders (FOD) are a subcategory of metabolic disorders that affect the functionality of fatty acid transport and mitochondrial β-oxidation (4). Complications due to fatty-acid oxidation disorders cause individuals to go into a metabolic crisis. This often requires hospitalization and emergency medical treatment. To prevent these crises, a screening program was implemented in 2002 in order to serve as an early detection system for these rare genetic disorders (1). This screening program identifies infants who are at risk of having a rare genetic disorder. Within the last five years an increase in false-positive results have been recorded by David Axelrod Institute, the center for the newborn screening program in New York. This study addresses this sudden increase in false-positive through a review of current procedures conducted and the analysis of past samples in order to determine if a secondary biomarker could be identified. Samples analyzed were from archival data preserved by New York State's Department of Health Division of Genetics at David Axelrod Institute. Results showed a statistically significant correlation, with an r-value of 0.887, between the levels of carbon fourteen (C14) and 3-hydroxypalmitoyl carnitine (C16:1-OH) in the blood of infants who were afflicted with glutaric-acidemia type two. Additionally the carbon eight (C8) biomarker was found to not be significantly correlated to the levels of C14 or C16:1-OH. These additional biomarkers are crucial in the differentiation of diagnosis between medium-chain acyl-CoA dehydrogenase deficiency and multiple acyl-CoA dehydrogenase deficiencies, which significantly decreases the risk of sudden unexpected death in infancy.

Literature Review

- Fatty-Acid Oxidation disorders are subcategories of metabolic disorders that affect the functionality of fatty acid transport and mitochondrial β-oxidation
  - (Houten et al., 2016).
- Rate of sudden unexpected death in infancy (SUDI) increased 32% from 1990-2000
  - Red Nose: Saving Little Lives
- Confirmed **FODs** were one of the **genetic causes of SUDI**
  - Lovera et al. 2003
- 2007 Congress enacted the Newborn Screening Saves Lives Act
- Tarini et al. 2011
  - Gestation age ***did impact*** newborn screening results (24)
  - Neonatal screening reduces incidences of metabolic crisis and death (17,18)
- New York State Department of Health found **a 152 false-positive** case increase from 2012 to 2016

Hypotheses



Significant **correlation *between*** screening results for biomarkers ***C14*** and ***C16*** in infants with ***MCADD and MADD will be found***

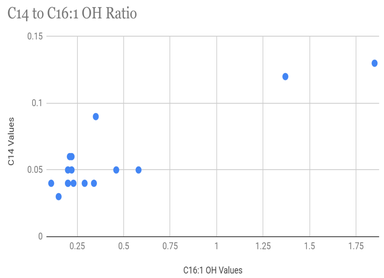
Methodology

- Step 1 • Heel prick test sent to lab within 48 hrs after birth
- Step 2 • Desired samples were hole punched to fit into well-plates
- Step 3 • 200 µL of Internal Standard were added
- Step 4 • Foil was placed over the samples
- Step 5 • Sample were incubated at 60℃ for 45 minutes
- Step 6 • 40 µL is extracted and placed into a new well plate
- Step 7 • 75 µL of butanolic HCl were added to the sample
- Step 8 • 200 µL of Internal Standard
- Step 9 • Incubated for 20 minutes at 60℃
- Step10 • 200 µL of reconstitution solution
- Step 11 • Foil was then placed on the well-plate
- Step 12 • Plates were placed into MS/MS
- Step 13 • Screening results were uploaded into NBS database

Data Analysis

Disorder	C8 Values	C 14 Vlaue	C16:1 OH Value
MADD	0.24	1.85	0.13
MCADD	1.58	0.11	0.04
MCADD	23.1	0.23	0.04
MCADD	16.09	0.22	0.06
MADD	0.066	0.58	0.05
MCADD	8.05	0.2	0.05
MCADD	10.91	0.15	0.03
MCADD	3.03	0.22	0.05
MCADD	4.05	0.2	0.04
MCADD	12.23	0.2	0.04
MADD	3.6	0.46	0.05
MADD	0.95	0.137	0.12
MCADD	1.85	0.35	0.09
MCADD	12.03	0.29	0.04

- Only individuals with Multiple Acyl-CoA dehydrogenase deficiency (MADD) displayed elevated levels of C14 and C16:1 OH.



- The correlation between the values of C14 and C16:1 OH in individuals with MADD was found to be statistically significant
- This test was indicative of a novel acylcarnitine biomarker that be implemented in the newborn screening system

Cross-Analysis With CLIR System

Collaborative Laboratory Integrated Reports (**CLIR**) is an interactive web tool created **Mayo Clinic** (17)

The clinical utility of CLIR is based on three major elements:

1. **Replacement** of traditional cutoff values with continuous adjustments for age and other covariates of reference ranges shown as seamless percentile charts.
2. **Creation** of cumulative, covariate-adjusted disease ranges for all informative markers for target conditions, usually clustered by specialty and/or type of markers
3. **Post-analytical interpretive** tools that integrate all relevant results into a single score to aid in diagnosis or prognosis of a condition

When compared to the Mayo Clinic's CLIR interactive web tool, there was no data to cross-reference the data collected regarding the possible C14 to C16:1 OH biomarker ratio

Conclusion

- The purpose of this research project was to improve the positive predicted value that is being used to identify true cases of MCAD deficiency. Additionally this project hopes to show the importance of research in this field in order to improve the lives of thousands of people worldwide. An experiment was conducted using archival blood samples from previous year's newborns screening tests. Samples were re-examined using a tandem mass spectrometer and the standard operating procedures of David Axelrod Institute. The hypothesis that a new biomarker would in fact be discovered upon analysis was supported. However the hypotheses regarding birth weight, gestation age, time elapsed between birth and heel prick test as well as gender were all disproved. The rejection of these hypotheses allow for greater understanding of how varying factors during and after childbirth do not necessarily affect the newborn screening test for MCAD deficiency and MADD. These novel discoveries can all be applied to the current methodologies used by David Axelrod Institute to conduct the newborn screens for New York State.

Acknowledgements

- Dr. Mark A. Morrissey: For your guidance over this project
- The Administration of NBS Department: Allowing me to conduct this research
- The NBS Lab Staff : Helping understand the process of NBS
- Ms. Gillian Rinaldo: Support and General Guidance
- My Science Research Peers: Aiding in the editing of my ppt, poster and paper
- My Parents & Grandma: Their never-ending support

Works Cited

1.Banta-Wright SA and Steiner RD. Tandem Mass Spectrometry in Newborn Screening A Primer for Neonatal and Perinatal Nurses. J Perinat Neonat Nurs 2004; 18(1): 41-5
2.Bartlett, K.; Eaton, S. Mitochondrial beta-oxidation. Eur. J. Biochem. 2004, 271, 462–469. [CrossRef] [PubMed]
3.Chace Dh, Kalas TA, Naylor EW. The application of tandem mass spectrometry to neonatal screening for inherited disorders of intermediary metabolism. Annual Rev Genomics Hum Genet 2002; 3:17-45
4.DeLonlay,P.;Giurgea,I.;Touati,G.;Saudubray,J.M.Neonatal Hypoglycaemia:Etiologies.Semin.Neonatol.2004,9,49–58. [CrossRef] [PubMed] Cells 2018,7, 46
5.DiMauro, S.; DiMauro, P.M. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. Science 1973,182, 929–931. [CrossRef] [PubMed]
6.Emery,J.L.;Howat,A.J.;Variend,S.;Vawter,G.F.Investigationofinbornerrorsofmetabolisminunexpected infant deaths.Lancet1988,2, 29–31. [CrossRef]
7.Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: E7720. https://doi.org/10.3897/BDJ.4.e7720. (n.d.). doi:10.3897/bdj.4.e7720.figure2f
8.Gregersen, N.; Lauritzen, R.; Rasmussen, K. Suberylglycine excretion in the urine from a patient with dicarboxylic aciduria.Clin. Chim. Acta 1976,70, 417–425. [CrossRef]
9.Houten, S.M.; Violante, S.; Ventura, F.V.; Wanders, R.J. The Biochemistry and Physiology of Mitochondrial Fatty Acid beta-Oxidation and Its Genetic Disorders. Annu. Rev. Physiol. 2016, 78, 23–44. [CrossRef] [PubMed]
10.Houten, S.M.; Wanders, R.J. A general introduction to the biochemistry of mitochondrial fatty acid beta-oxidation.J. Inherit. Metab. Dis.2010,33, 469–477. [CrossRef] [PubMed]
11.Karpati, G.; Carpenter, S.; Engel, A.G.; Watters, G.; Allen, J.; Rothman, S.; Klassen, G.; Mamer, O.A. The syndrome of systemic carnitine deficiency. Clinical, morphologic, biochemical, and pathophysiologic features. Neurology 1975,25, 16–24. [CrossRef] [PubMed]
12.Kelly, D.P.; Whelan, A.J.; Ogden, M.L.; Alpers, R.; Zhang, Z.F.; Bellus, G.; Gregersen, N.; Dorland, L.; Strauss, A.W. Molecular characterization of inherited medium-chain acyl-CoA dehydrogenase deficiency. Proc. Natl. Acad. Sci. USA 1990,87, 9236–9240. [CrossRef] [PubMed]
13.Kompare, M.; Rizzo, W.B. Mitochondrial fatty-acid oxidation disorders. Semin. Pediatr. Neurol. 2008,15, 140–149. [CrossRef] [PubMed]
14.Kotter's, M.; Jaksch, M.; Ketelsen, U.P.; Weiner, S.; Glocker, F.X.; Lucking, C.H. Valproic acid triggers acute rhabdomyolysis in a patient with carnitine palmitoyltransferase type II deficiency. Neuromuscul. Disord. 2001,11, 757–759. [CrossRef]
15.Mansouri,A.;Fromenty,B.;Durand,F.;Degott,C.;Bernuau,J.;Pessayre,D.Assessment The Prevalence Of genetic metabolic defects in acute fatty liver of pregnancy
J. Hepatol.1996,25, 781. [CrossRef]
16.Matsubara, Y., Narisawa, K., Tada, K., Ikeda, H., Yao, Y. Q., Danks, D. M., . . . McCabe, E. R. (1991, August 31). Prevalence of K329E mutation in medium-chain acyl-CoA dehydrogenase gene determined from Guthrie cards. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1678810
17.Matsubara, Y.; Narisawa, K.; Miyabayashi, S.; Tada, K.; Coates, P.M. Molecular lesion in patients with medium-chain acyl-CoA dehydrogenase deficiency.Lancet 1990,335, 1589. [CrossRef]
18.Marquardt G, Robert Currier R, McHugh DMS, et. al. Enhanced interpretation of newborn screening results without analyte cutoff values. Genetics in Medicine 2012; 14(7): 648-645
19.McHugh DMS, Cameron CA, Abdenu JE, et. al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: A worldwide collaborative project. Genetics in Medicine 2011: 13(3): 230-254.