| ID | 001-06 | 002-08 | 003-09 | 004-08 | 005-09 | 006-06 | 008- | 009- | 010- |
|---|---|---|---|---|---|---|---|---|---|
| Age (y) | 16 | 27 | 24 | 23 | 23 | 87 | 67 | 32 | |
| Age at diagnosis (y) | 1 | 15 | 12 | 10 | 14 | 78 | 62 | 22 | newborn |
| Sex | M | M | F | M | F | M | F | M | M |
| Genotype | c.338C>T (p.S113L) c.534_558 delinsT | c.338C>T (p.S113L) c.1784delC (p.P595GInfsX3) | c.338C>T (p.S113L) c.1784delC (p.P595GInfsX3) | c.338C>T (p.S113L) c.1369A>T (p.K457X) | c.338C>T (p.S113L) c.1369A>T (p.K457X) | c.149C>A (p.P50H) Unk | c.338C>T (p.S113L) c.338C>T (p.S113L) | c.338C>T c.1239_1240delGA (p.K414Tfs) | c.370C>T (p.R124X) c.370C>T (p.R124X) |
| Biochemistry (Average & SD, μM) | | | | | | | | | |
| C0 | 35.0 (6.5) | 57.0 (42.7) | 28.5 (0.7) | 40.0 (14.8) | 25.9 (10.0) | 73.4 (23.5) | 46.0 (8.4) | 47.8 (12.9) | NA |
| C2 | 7.51 (5.88) | 11.38 (14.93) | 5.20 (1.42) | 5.29 (2.34) | 4.62 (1.90) | 16.50 (9.30) | 12.0 (5.1) | 5.73 (2.54) | NA |
| C14:1 | 0.12 (0.15) | 0.11 (0.06) | 0.10 (0.05) | 0.07 (0.05) | 0.13 (0.10) | 0.15 (0.10) | 0.09 (0.01) | 0.10 (0.08) | NA |
| C16 | 0.58 (0.40) | 0.51 (0.27) | 0.48 (0.31) | 0.32 (0.13) | 0.44 (0.17) | 0.85 (0.28) | 0.45 (NA) | 0.55 (0.23) | NA |
| C16:1 | 0.08 (0.07) | 0.10 (0.07) | 0.04 (0.02) | 0.05 (0.02) | 0.08 (0.05) | 0.09 (0.06) | 0.06 (NA) | 0.08 (0.05) | NA |
| C18 | 0.28 (0.20) | 0.21 (0.09) | 0.27 (0.09) | 0.18 (0.10) | 0.21 (0.10) | 0.41 (0.09) | 0.20 (NA) | 0.24 (0.08) | NA |
| C18:1 | 0.66 (0.64) | 0.64 (0.35) | 0.35 (0.09) | 0.39 (0.22) | 0.63 (0.35) | 0.74 (0.34) | 0.46 (NA) | 0.64 (0.47) | NA |
| C18:2 | 0.31 (0.26) | 0.31 (0.12) | 0.17 (0.03) | 0.17 (0.10) | 0.30 (0.19) | 0.34 (0.22) | 0.21 (NA) | 0.28 (0.16) | NA |
| CK | 540 (1655) | 43869 (102970) | 75 (7) | 178 (75) | 4052 (10756) | 270 (97) | 1975 (3727) | 228 (219) | NA |
| ALT | 45 (38) | 295 (545) | 18 (1.4) | 29 (21) | 119 (265) | 63 (97) | 78 (78) | 41 (21) | NA |
| AST | 69 (90) | 1389 (2732) | 26.5 (9) | 45 (30) | 141 (314) | 46 (41) | 177 (222) | 39 (17) | NA |

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## ePO32

### Measurement of Nicotinamide Adenine Dinucleotide (NAD+) from dried blood spot cards

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**Introduction:** Within the human body, all cells, from our neurons to our skin, require an energy source for function.
ATP is the cellular energy source and is generated by mitochondria through oxidative phosphorylation.
Nicotinamide adenine dinucleotide (NAD+) serves as an essential cofactor in the generation of ATP by the mitochondria. Due to this role in energy metabolism, measurement of NAD+ can provide insight into overall health and well-being. It is also a helpful tool in identifying supplementation needs and monitoring effectiveness.
Previously, we demonstrated that NAD+ could be effectively stabilized on a chemically treated DBS card. This novel method was validated and the normal range was found to be consistent with reported ranges from methods that measured directly from whole blood. We have since expanded this work to continue to establish the validity of this approach and to explore the potential for at home sampling.
**Methods:** Three different experiments were designed for this study. The first was a patient matched study to allow for direct comparison of NAD+ measurements from the DBS method and a generally accepted whole blood-based method offered by NADmed (Helsinki, Finland). Twelve donors participated in the study. A dried blood spot and venous drawn whole blood (EDTA) sample was collected from each donor. DBS samples were analyzed within 48 h of collection. Whole blood samples were placed at $\leq -80$ °C immediately following collection and shipped on dry ice for analysis.
Next, dried blood spot samples were collected from six individuals. Samples were analyzed after drying and then stored at room temperature (15 – 30 °C) and $\leq -20$ °C. NAD+ measurements were taken at various timepoints to establish stability. All measurements were compared back to the t=0 values.
Finally, efforts have been made to refine the chemical treatment of DBS cards to determine if the stability of NAD+ could be further improved. DBS samples were collected from 11 donors on two sets of treated cards. The first set contained the original treatment and the second was an optimized coating. NAD+ measurements from each card type were compared.
**Results:** For the novel dried blood spot method, the average measured NAD+ concentration from the 12 donors was 24.81 μM with a range spanning 19.89 – 29.30 μM. The existing NADmed method resulted in an average of 25.85 μM with a range of 20.36 – 32.13 μM. The average difference between the two methods was 4.9%.
The DBS stability study revealed that when stored at room temperature, 83% of NAD+ measurements taken after 7 days of storage were still consistent with the initial result. Storing at $\leq -20$ °C was found to significantly increase stability to 6 months of storage.
The original card treatment resulted in an average NAD+ concentration of 27.74 μM across the 11 donors, with a range of 24.14 – 32.19 μM. For the optimized treatment, the average measurement was 34.08 μM with a range of 29.16 – 39.42 μM. In all cases, a higher NAD+ measurement was obtained from the optimized coating compared with the original (average increase was 18.5%).

**Conclusion:** The results of this study confirmed that NAD+ measurements from DBS are comparable to a previously validated whole blood method. The chemically treated DBS cards provide acceptable stability to allow for at home sampling, reasonable shipping conditions, and potential for sample batching within the laboratory. Optimization of the chemical coating further stabilizes NAD+ within the DBS card and thereby improves the robustness and quality of the assay.

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## ePO33

### COASY-associated pontocerebellar hypoplasia – A possible additional secondary target detectable by expanded newborn screening?

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**Background:** Carnitine palmitoyl transferase I (CPT I; OMIM: 255120) deficiency is an autosomal recessive disorder that impairs long-chain fatty acid transport into the mitochondria, subsequently leading to isolated elevations in free carnitine and a reduction in long chain fatty acids. CPT I deficient patients typically present with heart failure, hypotonia, hepatic abnormalities, and hypoketotic hypoglycemia during fasting periods. Although clinically similar to other long chain fatty acid oxidation disorders, CPT I deficiency was initially thought to have a unique metabolic derangement. The latter has recently been challenged by individuals presenting with apparently identical biochemical abnormalities, yet without pathogenic variants in the expected gene. Contrarily, these patients harbor variants in the *COASY* gene which encodes for a dual functioning enzyme responsible for the final steps (4-phosphopantethine adenyl transferase and dephospho-CoA kinase) of coenzyme A synthesis. Biallelic pathogenic variants in *COASY* may result in severe hypotonia, episodes of dystonia, pontocerebellar hypoplasia, and/or neurodegeneration via iron accumulation in the brain, among other. Considering the heterogenous clinical presentation of previously reported cases, as well as the indistinguishable biochemical presentation of CPT I and *COASY*-related disorders, these diseases clearly provide a diagnostic challenge.
**Case presentation:** Two female siblings presenting with hyperglycemia, severe hypotonia, and respiratory insufficiency were admitted to the University of Rochester Medical Center neonatal intensive care unit, on two separate occasions. Upon examination, evidence of poor Moro and deep tendon reflexes, along with a resting 'frog-legged' appearance, were observed. Initial brain magnetic resonance imaging (MRI) of the siblings revealed symmetric areas of diffusion restriction in the bilateral hippocampi, globus pallida, thalami, and posterior limbs of the internal capsule suggestive of hypoxic injury or metabolic disease. Subsequent MRI in the older sibling revealed progressive atrophy of the cortical and brainstem structures consistent with pontocerebellar hypoplasia. Although the newborn screening and confirmatory plasma acylcarnitines/carnitine results of these patients were suggestive of CPT I deficiency, molecular testing excluded this inborn error of metabolism. Nonetheless, exome sequencing revealed homozygous variants of uncertain significance in the *COASY* genes of both siblings, suggesting a possible COASY-related disease, even though clinical abnormalities did not correlate with previously reported cases. When comparing the biochemical data of these patients to previously confirmed CPT I patients using Collaborative Laboratory Integrated Reports web application (CLIR; https://clir.mayo.edu), the ratios of markers not currently considered by standard newborn screening appeared to vary notably between these diseases.
**Conclusion:** *COASY*-related disorders may be indistinguishable from CPT I by newborn screening and conventional post-analytical interpretation. However, utilizing CLIR, we identified metabolite marker ratios that may differentiate between these diseases.

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## ePO34

### Novel use of global untargeted metabolomics in a patient with glycogen storage disease Ib receiving off label empagliflozin treatment

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**Background:** Glycogen storage disease type Ib (GSD-Ib) is a rare inborn error of glycogen metabolism. Affected individuals present with fasting intolerance, severe hypoglycemia, hepatomegaly, and lactic acidosis. The disorder is uniquely associated with neutropenia and neutrophil dysfunction causing serious infections, inflammatory bowel disease (IBD), mucosal lesions, and impaired wound healing. Recently, kidney sodium-glucose co-transporter-2 (SGLT2) inhibitors such as empagliflozin, known to reduce plasma levels of 1,5-anhydroglucitol (1,5-AG) and its toxic derivatives in neutrophils, have been described as a new treatment option in case reports of patients with GSD-Ib from Europe and Asia.
**Case presentation:** We hereby report our experience with an 11-year-old girl with GSD-Ib presenting with short fasting hypoglycemia, neutropenia with neutrophil dysfunction, recurrent infections, suboptimal growth, iron-deficiency anemia, recurrent abdominal pain, and loose stools. Treatment with daily empagliflozin resulted in improvement in neutrophil counts and function, leading to resolution of recurrent infections and mouth sores with significant reduction in G-CSF needs. Significant improvement in IBD symptoms with normalization of inflammatory markers and bowel imaging has led to weight gain