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

with improved nutritional markers. Furthermore, improvement of IBD symptoms allowed spacing of meals and cornstarch doses and improved fasting intolerance. Reduction of maximum empagliflozin dose was needed due to arthralgia. No other significant side effects of empagliflozin were observed, including hypoglycemia. This report also highlights novel use of global metabolomics for monitoring plasma levels of 1,5-anhydroglucitol to assess empagliflozin dose responsiveness and guide dietary management as well as G-CSF therapy. Clinical improvement correlated to rapid normalization of 1,5-AG levels in plasma that was sustained after dose reduction.

Conclusion: SGLT2 inhibitors are a new and safe treatment option for GSD-Ib-associated neutropenia and neutrophil dysfunction leading to overall improvement in other manifestations of the disease. Global untargeted metabolomics is an efficient method to assess biochemical responsiveness to treatment.

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eP035

Genotype-phenotype correlation of glycogen storage disease type IV

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Introduction: Glycogen storage disease type IV (GSD IV) is a rare autosomal recessive disease caused by glycogen branching enzyme deficiency, encoded by GBE1. The deficiency results in variable clinical features, age of onset, and disease severity. GSD IV is arbitrarily divided into several subtypes based on the continuum of symptoms. The most common Classic Hepatic subtype presents in the first few months of life with hepatomegaly, followed by rapid deterioration into cirrhosis and lethal liver failure requiring liver transplant. The Non-progressive Hepatic subtype is thought to be rare and can present with hepatomegaly in childhood with or without myopathy and cardiomyopathy, with those affected surviving into adulthood. The most severe Fetal/Neonatal Neuromuscular subtype can present with fetal akinesia, hypotonia, dilated cardiomyopathy, and death in early infancy. The Congenital Neuromuscular subtype presents in the newborn period with profound hypotonia, respiratory distress, and typically with dilated cardiomyopathy, that can lead to death in childhood. A milder Childhood Neuromuscular subtype can lead to myopathy or cardiomyopathy in late childhood. Certain genotypes in GBE1 are associated with Adult Polyglucosan Body Disease (ABPD), characterized by adult-onset neuropathy, spasticity, autonomic dysfunction, and mild cognitive decline. Recently, Isolated Cardiomyopathy subtype was described in a patient with biallelic GBE1 missense variant. The diagnosis of GSD IV is challenged by the variable presentation and confounding histology, even within the same family. At initial early presentation, the classic hepatic subtype can be indistinguishable from the non-progressive hepatic phenotype, yet liver transplantation arrangement should be initiated rapidly after the diagnosis. Early and accurate diagnosis of hepatic phenotypes is critical in enlisting the patient for liver transplant candidacy. However, previous reviews of GSDIV genotype-subtype associations have not addressed the prediction of Hepatic phenotypes based on GBE1 genotype. We performed a comprehensive review of case reports and case series of the spectrum of glycogen branching enzyme deficiency of all subtypes to analyze genotype-phenotype correlation.

Methods: An independent English literature search of the PubMed and Google Scholar databases was performed, using the keywords "glycogen storage disease", "adult polyglucosan body disease" "GBE", and "GBE1". The search was performed with no time restrictions for publication year. Manual inspection of the manuscripts was conducted to include published case reports and case series describing clinical features and outcomes of patients with GSD IV. Studies were excluded if they did not describe clinical patient data, if the diagnosis was not confirmed by enzyme activity/GBE1 sequencing or if the patient was previously described in the published literature. After studies were reviewed for inclusion, data extraction was performed. Author name, bibliographic details, number of patients, patient sex, age of disease onset, the clinical presentation of disease with specific organ involvement, and clinical outcomes were recorded. Each reported case was classified into one of the six GSD IV subtypes described above, based on patients' presenting symptoms described in the respective case report. GBE1 genotypes were recorded, if available.

The GBE1 variant descriptions were standardized according to the HGVS nomenclature. Variant Validator https://variantvalidator.org/ was used to verify the DNA nomenclature. GBE1 genotypes for cases in each subtype were analyzed.

Results: One hundred sixty-eight cases from 99 publications were selected, which reported 132 variants in the GBE1 gene. The cases consisted of 33 cases of Classic Hepatic subtype, 20 cases of Non-progressive Hepatic subtype, 54 cases of Fetal/Neonatal Neuromuscular subtype, 7 cases of Congenital Neuromuscular, 16 cases of Childhood Neuromuscular subtype, 26 cases of APBD subtype and 1 case of Isolated Cardiomyopathy. Ten cases did not include sufficient clinical information to enable classification into one of the seven subtypes.

All Classic Hepatic subtypes with known genotype had one severe variant (splice-site, non-sense, frameshift) in compound heterozygosity with a missense variant. The mild variant p.Tyr329Ser commonly seen in APBD was also commonly seen in Non-Progressive Hepatic subtype in compound heterozygosity with a sever variant (missense variant leading to complete loss of the GBE activity or splice site variant).

We were not able to find genotypes characteristic to either Hepatic subtype. Prediction of the Hepatic subtypes based on the GBE1 genotype alone remain difficult. Non-sense variants and splice-variants were seen in both subtype in compound heterozygosity with a missense variant. Large exonic variants were not found in either Hepatic subtype. Variants found in both subtypes are similarly distributed across the gene with no specific common hot spots.

Having at least one truncation variants due to a large deletion, non-sense, frameshift or splice-site variants have been implicated in the severe Fetal/Neonatal Neuromuscular subtype and we confirmed this finding.

We found that Fetal/Neonatal Neuromuscular subtype was most frequently reported, contrary to the previous report that the Classic Hepatic subtype is the most common subtype. Publication biases likely play a role in reporting the more severe phenotype.

Our work adds to the previous reviews on GSD IV genotypes, along with a recent review on APGD, especially for the updated genotype description according to the HGVS nomenclature.

Conclusion: The current literature regarding glycogen storage disease type IV is limited; however, multiple studies suggest a potential link between genetic variation within the GBE1 gene and disease severity as well as timing of symptoms. Our current study shows that the most severe and frequently reported subtype, Fetal/Neonatal Neuromuscular, is associated with multiple truncation variants. Although these findings are limited by the rarity of the disease, this work provides an in-depth review of genotype-phenotype correlation in glycogen storage disease type IV. Further studies are warranted to better understand the impact that genetic variations play in disease presentation and outcomes.

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