PRECISION MEDICINE

Metabolon

TRANSPORTER AND COFACTOR DEFICIENCIES

Metabolites are the small molecule products and intermediates of metabolism onto which inputs from the genome, environment, and lifestyle factors converge, making them perhaps the closest reflection of the real-time health status of an individual.

Metabolites reflect disease activity through changes in their abundance, which can be measured with ultra-high performance liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS) technologies. When used in an untargeted manner, UPHLC-MS/MS can measure the entire collection of metabolites in a given biological matrix to obtain a broad biochemical profile of an individual. This metabolic profile can identify metabolic perturbations to diagnose or inform on disease. Metabolomics has proven to be a powerful tool for gaining deep phenotypic insight into disease, including inborn errors of metabolism (IEMs).

IEMs are genetic disorders that impair the production and/or activity of enzymes, transporters, or cofactors required for normal metabolism. Most IEMs have serious clinical consequences if left untreated, which underscores the importance of early diagnosis. More than 500 IEMs have been described yet for most of them, diagnostic tests remain unavailable. Furthermore, a large number of IEMs present with an undifferentiated clinical phenotype, which typically subjects affected individuals to a diagnostic odyssey, delaying the start of treatment and potentially resulting in life-long health complications. Using its untargeted metabolic profiling platform, Metabolon has made significant contributions towards expanding the ability to diagnose IEMs, characterizing undifferentiated IEM phenotypes, and identifying metabolite biomarkers that may facilitate treatment monitoring. Here, we summarize our most important work in transporter and cofactor deficiencies.

Early Infantile Epileptic Encephalopathies (EIEE)

EIEE are characterized by refractory seizures that result in cognitive, sensory and motor abnormalities. EIEE can be caused by structural brain malformations, inborn errors of metabolism (IEM), injury, or genetics. SLC13A5 is a tricarboxylate substrate transporter expressed in liver and brain. Mutations in the SLC13A5 gene is one cause of EIEE25, yet the underlying causal mechanism is unknown. To better understand the metabolic perturbations associated with this condition we performed metabolic profiling on cerebrospinal fluid (CSF), plasma, and urine collected from 5 patients with confirmed loss of function variants of SLC13A5 [1].

Compared to healthy controls, 3, 4, and 25 metabolites were consistently and significantly perturbed in urine, plasma, and CSF, respectively. In CSF and plasma citrate,

an important substrate for energy production, was significantly increased compared to controls but was unchanged in urine. Other TCA cycle intermediates were also perturbed in urine (fumarate) and CSF (isocitrate, 2-methylcitrate & aconitate). 25 metabolites were altered in the CSF, 6 of which were involved directly with amino acid metabolism. 2-methylcitrate was increased by approximately 4-fold in patient samples while 3-hydroxybutyrate (BHBA) was significantly decreased. Distinctive elevations of citrate and dysregulation of citric acid cycle intermediates support the hypothesis that loss of SLC13A5 function alters tricarboxylic acid cycle metabolism. Given the potential for astrocytes to modulate neuronal excitability, astrocyte dysfunction in SLC13A5 deficiency may be a key component of the pathophysiologic mechanism underlying the epileptic phenotype.

We note that although genome wide sequencing can provide a definitive diagnosis of EIEEs it is expensive, has a long turnaround time, and poor rates of third-party reimbursement. Clinically available plasma organic acid testing can directly detect increased plasma citrate but does not detect most TCA metabolites. Interrogating the metabolome provides a less expensive and more expeditious alternative method of investigating potential diagnoses and underlying mechanisms of IEMs.

GLUT1 deficiency syndrome (GLUT1-DS)

GLUT1-DS is the inherited deficiency of glucose transporter type 1 (GLUT1), the transporter responsible for shuttling glucose across the blood brain barrier. Low levels of glucose in the brain results in hypoglycorrhachia and impaired function of the neurons in the superficial layers of cerebral cortex, hippocampus, basal ganglia, and thalamus. GLUT1-DS can be treated with a ketogenic diet, and early treatment is associated with a better prognosis. However, it is not routinely tested by newborn screening, and the ketogenic diet has been shown to lose its therapeutic efficacy in ~20% of cases. In one study [2] we aimed to use metabolomics as a biomarker discovery tool to identify novel markers associated with GLUT1-DS and ketogenic diet therapy. The long term goal of this work is to expand our diagnostic capability for the disease and develop alternative, second line therapies.

We performed untargeted metabolic profiling on CSF collected from patients with GLUT1-DS being fed a ketogenic diet. The most affected super pathway was lipid metabolism with long chain fatty acid, phospholipid, acylcarnitine and sphingolipid metabolites showing significant disturbance in GLUT1-DS patients on ketogenic diet. Relative to healthy controls, GLUT1-DS patients on a ketogenic diet demonstrated elevated acylcarnitines linked to fatty acid metabolism and lower levels of amino acid-related acylcarnitines, 2-methylmalonyl carnitine, isovalerylcarnitine, propionylcarnitine, isobutyrylcarnitine, glutarylcarnitine, carnitine and 2-methylbutyrylcarnitine. 3-hydroxybutyrylcarnitine, oleoylcarnitine, stearoylcarnitine were significantly higher in patients. As expected 3-hydroxybutyrate (BHBA) was also significantly elevated in plasma.

Currently, the routine diagnostic tools in clinical use for GLUT1-DS are low glucose in CSF and a reduced CSF glucose to blood glucose ratio. Glucose values can vary depending on age and screening for glucose levels versus a pediatric-matched population can identify perturbations in metabolites, such as carbohydrate metabolism, to identify signatures of disease. This study demonstrates how applying metabolomics to clinical practice could contribute an informative diagnosis and provide insight into the pathophysiological mechanisms of IEMs to broaden therapeutic choices.

Riboflavin transporter deficiency (RTD)

RDT is a neurogenetic disorder that most commonly manifests as hearing loss, peripheral neuropathy, respiratory insufficiency, and bulbar palsy. Individuals with RTD reportedly have an acylcarnitine profile that resembles a different IEM: acyl-CoA dehydrogenase deficiency. This shared biochemical phenotype has been linked to inadequate bioavailability of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN),

riboflavin metabolites that are important for normal functioning of the mitochondrial respiratory chain. Here [3], we used untargeted metabolic profiling to evaluate the biochemical profile and therapeutic efficacy of a riboflavin supplementation diet on a 2-year-old-boy.

The patient presented with developmental regression, and the uncharacteristic symptoms of macrocytic anemia and intermittent neutropenia. Whole exosome sequencing confirmed a diagnosis of RTD. A baseline plasma sample was taken before the patient started riboflavin supplementation. Plasma samples were taken periodically after supplementation to monitor the effect of treatment. Vitamin B6 levels were restored in response to treatment, which is consistent with increased availability of FMN. Several other metabolic pathways including tryptophan metabolism, sulfur amino acid metabolism, and one carbon metabolism also normalized. In the baseline sample multiple medium and very long chain acylcarnitines were elevated. These too, normalized following riboflavin supplementation. Normalization of the patient's biochemical profile was reflected by resolution of his anemia, neutropenia, and neurological symptoms.

Recovery of the patient's anemia and intermittent neutropenia with riboflavin supplementation supports a clinical expansion in the known phenotype of RTD. This finding also shows that a young child with macrocytic anemia, neutropenia, and neurological manifestations should receive riboflavin supplementation, regardless of the plasma level, while awaiting genetic and biochemical workup. Untargeted metabolomics analysis provided a more comprehensive pattern of biochemical perturbations associated with RTD, which supports the role of riboflavin requiring enzymes in these metabolic processes.

NAXE deficiency: a nurometabolic disorder of NAD(P)HX repair amenable for metabolic correction

The gene NAXE encodes an enzyme that facilitates intracellular NAD+ metabolism and efflux of cholesterol from endothelial cells and macrophages into high density lipoprotein particles (HDL). Biallelic loss of function variants in NAXE cause severe progressive infantile encephalopathy with brain edema, leukoencephalopathy, and pellagra-like skin lesions. None of the cases published to date report abnormal laboratory findings capable of serving as disease-specific biomarkers. In virtually all cases, diagnosis was made by exome sequencing (ES).

NAXE is an essential component of the NAD(P)HX repair system in humans, as reflected by the severe neurometabolic and neuroinflammatory condition, PEBEL1, associated with its deficiency. While the co-occurrence of pellagra-like skin lesions, encephalopathy, and brain edema triggered by febrile illness may strongly suggest PEBEL1, further case identification has demonstrated a broader phenotypic spectrum, including cases with nonspecific clinical and neuroimaging findings.

To characterize these findings we performed untargeted metabolomic profiling on individuals with NAXE deficiency [4]. Results showed a complex picture of the breadth of abnormalities, derived from reduced cellular NAD+/NADH/NAD(P)H but also associated with NAD(P)HX epimerization and binding of apolipoproteins. Supplementation with niacin appears to replete NAD+ stores, suggesting niacin as a preventative measure to alleviate crises. However, longer periods of follow-up are needed to provide more information on the natural history of the disorder in undulating cases and treated patients experiencing febrile illnesses.

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Biallelic variants in SLC38A3 encoding a glutamine transporter cause epileptic encephalopathy

The solute carrier (SLC) family of proteins is a superfamily of transmembrane transporters that include more than 400 members, which are involved in the exchange of amino acids, nutrients, neurotransmitters, and metabolites across biological membranes. More than 280 SLC genes are expressed in the brain and play a crucial role in energy metabolism and synaptic vesicle and neurotransmitter release. Even though defects in several SLC-encoding genes have been linked to various developmental and epileptic encephalopathies (DEE), 45-70% of DEE cases are not given a conclusive molecular diagnosis because the availability of genetic testing is variable and the molecular yield of DEE varies significantly by age of onset. Even if a mutation is directly linked to a patient's DEE, often times the resulting biological dysfunction remains uncharacterized.

To better understand underlying pathophysiology of deleterious SLC variants Metabolon performed untargeted metabolic profiling on plasma, urine, and cerebrospinal (CSF) samples collected from 10 individuals with DEE from seven unrelated families from six different countries who had molecular-confirmed biallelic predicted-damaging variants in SLC38A3 [5]. SNAT3 is a member of the SLC superfamily involved in urea formation in the liver, pH regulation in the kidney, and insulin secretion in the pancreas [6]. Findings revealed that urea was consistently low in all available samples and plasma ammonia was elevated in four subjects consistent with SNAT3 not providing glutamine for urea formation. One patient demonstrated significantly elevated N-acetylglutamine, N-acetylasparagine, and 1-palmitoylglycerol and significantly low levels of aspartate and cysteine in the CSF. The same patient had undetectable levels of N-acetylglutamine in plasma. Plasma glutamine and N-acetylglutamine was significantly low in this patient's affected sibling. Altogether, the abnormalities in glutamate metabolism may indicate the role for SNAT3 across the blood-brain barrier and potentially represent metabolic markers of the disease.

References

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