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Novel biomarkers of succinic semialdehyde dehydrogenase deficiency highlight opportunities for screening and detection of GABA catabolism pathway abnormalities

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Introduction: GABA (gamma-aminobutyric acid) is catabolized in the mitochondrial matrix via the GABA shunt, a 2-step enzymatic pathway which encompasses the transamination of GABA to succinic semialdehyde by GABA-transaminase (GABA-T), followed by oxidation to succinate by succinic semialdehyde dehydrogenase (SSADH). Deficiency of SSADH results in accumulation of GABA and reduction of succinic semialdehyde to gamma-hydroxybutyrate (GHB), the principal neurotoxin in SSADH deficiency (SSADHD). SSADHD is an autosomal recessive disorder associated with biallelic pathogenic variants in *ALDH5A1,* typically characterized by developmental delay, hypotonia, intellectual disability, ataxia, seizures, hyperkinetic behavior, aggression, psychiatric disorders, and sleep disturbances. The clinical spectrum and severity of symptoms are variable, and genotype-phenotype correlations have not been observed, hampering clinical suspicion. Therefore, diagnosis is often delayed. Disease incidence is estimated to be about 1:460,000; however, given the lack of specific clinical features, this is likely an underestimation of prevalence. SSADHD is diagnosed by elevated GHB by urine organic acid analysis in suspected patients. However, not all clinical diagnostic laboratories routinely report this biomarker, and targeted diagnosis in blood is not clinically available. To improve diagnostic capability and to develop cost-effective, efficient approaches to screening that may also be integrated into newborn screening technology platforms, blood-based biomarkers are required. Here, we describe the clinical features and metabolomic findings for two patients with SSADHD and define new biomarkers in plasma that will improve diagnostic specificity for this condition.

Methods: Patients A and B are full siblings, sister and brother, both with confirmed SSADHD due to elevated urine GHB (>500 mmol/mol creatinine) and biallelic variants in *ALDH5A1,* c.612G>A (p.Trp204Ter) and c.1234C>T (p.Arg412Ter). Human genome build GRCh37/hg19 and NCBI reference sequence NM_001080.3 were used for all molecular analyses in this study.

Patient A had early developmental delays, loss of babbling, decreased interest in interactive play, and unusual hand postures. MRI of the brain at 12 months showed subtle bilateral increased signaling in the globus pallidus and head CT at 20 months, performed because of vomiting, head circumference of 95th percentile, and developmental delay, showed benign macrocephaly of childhood. Developmental milestones were delayed; she sat unsupported at 11 months, crawled at 14 months, and walked at 2 years. At 2.5 years, visual perception and fine motor skills were below average, receptive language was average, and while non-verbal, the patient communicated using sign language. Diagnosis of SSADHD was confirmed at 27 months of age with urine organic acids (UOA) showing >500 mmol/mol creatinine elevation of GHB and confirmed by targeted sequencing of *ALDH5A1*. Chromosome microarray, karyotype, other metabolic testing, and EEGs were all normal/non-diagnostic. At age 12 years, she speaks, but has difficulty with articulation, has normal vision and hearing, and is a happy and social child. She has only been hospitalized once as a toddler for acute gastroenteritis that required IV fluid hydration.

Patient B, younger brother of Patient A, was diagnosed with SSADHD at 4.5 months, 1.5 months after his sister's diagnosis, with findings from UOA analysis and *ALDH5A1* sequencing consistent with his sister's diagnosis. His brain MRI showed very subtle increased T2 signal in the globus pallidus bilaterally and a normal MR spectroscopy. He had normal muscle tone for the first 4 months of life, rolled over and lifted his head on time and sat by 6 months. However, he started missing developmental milestones, with hypotonia noted by the age of 6 months. He began to babble at 12 months, crawled at 13 months, and walked at 2.5 years old. At age 10, he has about 5 words, uses sign language as his primary form of communication, can sign 4-5-word sentences, and uses a computer tablet to create sentences through pictures.

Neither sibling has seizures, aggression, or self-injurious behaviors, but both have ADHD, OCD, and behaviors of perseveration and fixation and exhibit tactile stimulation, with the sister hand flapping and the brother bouncing. At younger ages, both siblings developed frequent night wakings, increased daytime sleepiness, and difficulty initiating sleep; however, these concerns abated with melatonin treatment. Neither patient takes other medications or supplements. Both children are non-dysmorphic, ambulatory, eat all food by mouth, and have high emotional IQ, with the ability to describe and explain if and why they are frustrated. Each child has had physical, occupational, speech, and equine therapies, and both continue to gain developmental skills.

Clinical untargeted metabolomic profiles were generated from plasma derived from venous whole blood obtained from each child. Semiquantitative analysis of metabolites in each sample was achieved by comparing the individual patient sample to a set of invariant anchor specimen included in each batch. Raw spectral intensity values were then normalized to the anchor samples, log transformed, and compared to a normal reference population to generate Z-scores.

Results: Analysis of >900 plasma metabolites, encompassing amino acids, neurotransmitters, lipids, carbohydrates, cofactors/vitamins, purines, and pyrimidines revealed significant elevations of 2-pyrrolidinone (Z-scores +4.80, +3.38) and 4-guanidinobutanoate (Z-scores +4.03, +3.53). Argininate, another guanidino-metabolite, was also modestly elevated (Z-scores +2.46, +2.93) in samples from both patients. These findings were confirmed by review of plasma metabolomic profile data from an unrelated patient with SSADHD (*ALDH5A1*: c.1597G>A (p.Gly533Arg) and c.1015-2A>C) that showed elevated 2-pyrrolidinone (Z-score +3.04) and 4-guanidinobutanoate (Z-score +3.20). Other mild lipid and amino acid alterations were observed in all 3 patient samples; however, the composition and degree of these alterations are not specific to SSADHD.

Conclusion: A previous metabolomic analysis of brain on autopsy from an adult with SSADHD showed elevations of guanidino-compounds and alteration of GABA, glutamine, and creatine levels, while another study also reported findings from dried blood spots including altered short-chain acylcarnitines, creatine, and ornithine. In the current study, untargeted metabolomic profiling of plasma showed significant elevations of 2-pyrrolidinone and 4-guanidinobutanoate, potential new biomarkers of SSADHD. We have previously shown that 2-pyrrolidinone, a lactam cyclization product of GABA, is an elevated, reliable biomarker of GABA-transaminase deficiency, which is consistent with the clinical significance of our current observations. Metabolomic profiling of a larger cohort of patients with SSADHD will determine the specificity and sensitivity of these biomarkers and will assess utility for newborn dried blood spot analysis. Correlation of metabolomic data with clinical profiles may also provide insight regarding variations associated with genotype, phenotype, demographics, medications, and nutritional status.

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