Pediatric Neurology 75 (2017) 66–72

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Original Article

Clinical Metabolomics to Segregate Aromatic Amino Acid Decarboxylase Deficiency From Drug-Induced Metabolite Elevations

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ABSTRACT

BACKGROUND: Phenotyping technologies featured in the diagnosis of inborn errors of metabolism, such as organic acid, amino acid, and acylcarnitine analyses, recently have been supplemented by broad-scale untargeted metabolomic phenotyping. We investigated the analyte changes associated with aromatic amino acid decarboxylase (AADC) deficiency and dopamine medication treatment. **METHODS:** Using an untargeted metabolomics platform, we analyzed ethylenediaminetetraacetic acid plasma specimens, and biomarkers were identified by comparing the biochemical profile of individual patient samples to a pediatric-centric population cohort. **RESULTS:** Elevated 3-methoxytyrosine (average *z* score 5.88) accompanied by significant decreases of dopamine 3-*O*-sulfate (–2.77), vanillylmandelate (–2.87), and 3-methoxytyramine sulfate (–1.44) were associated with AADC deficiency in three samples from two patients. In five non-AADC patients treated with carbidopa-levodopa, levels of 3-methoxytyrosine were elevated (7.65); however, the samples from non-AADC patients treated with DOPA-elevating drugs had normal or elevated levels of metabolites downstream of aromatic L-amino acid decarboxylase, including dopamine 3-*O*-sulfate (2.92), vanillylmandelate (0.33), and 3-methoxytyramine sulfate (5.07). In one example, a plasma metabolomic phenotype pointed to a probable AADC deficiency and prompted the evaluation of whole exome sequencing data, identifying homozygosity for a known pathogenic variant, whereas whole exome analysis in a second patient revealed compound heterozygosity for two variants of unknown significance. **CONCLUSIONS:** These data demonstrate the power of combining broad-scale genotyping and phenotyping technologies to diagnose inherited neurometabolic disorders and suggest that metabolic phenotyping of plasma can be used to identify AADC deficiency and to distinguish it from non-AADC patients with elevated 3-methoxytyrosine caused by DOPA-raising medications.

Keywords: inborn error of metabolism, biochemistry, neurotransmitter, metabolomics, dopamine, aromatic amino acid decarboxylase deficiency, phenotype, diagnosis

Pediatr Neurol 2017;75:66–72

Introduction

Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM #608643) is a rare genetic disorder that disrupts the conversion of tyrosine and tryptophan into neurotransmitters and catecholamines,[1] and while a pan-ethnic disorder, it is most commonly observed in southern Chinese populations.[1,2] Mutations in the *DDC* gene inhibit the conversion of the tyrosine biochemical L-3,4-dihydroxyphenylalanine (L-DOPA) into dopamine, and the deficiency inhibits the conversion of the tryptophan metabolite 5-hydroxytryptophan into serotonin. Decreased levels of dopamine, its catecholamine derivatives norepinephrine and epinephrine, and serotonin lead to symptomatic features of AADC deficiency including developmental delay, hypotonia, athetosis, failure to thrive, dystonia, oculogyric crises, and autonomic and neurological dysfunction.

Article History:
Received May 10, 2017; Accepted in final from June 26, 2017
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0887-8994/$ — see front matter
http://dx.doi.org/10.1016/j.pediatrneurol.2017.06.014

Traditional diagnostic avenues for AADC deficiency include enzymatic testing of AADC, neurotransmitter profiling of cerebrospinal fluid (CSF), and molecular testing of *DDC* for pathogenic variants. Biomarkers of AADC deficiency include elevated 3-methoxytyrosine (3-MT, 3-*O*-methyldopa), reduced homovanillic acid, and 5-hydroxyindoleacetic acid in the CSF. Elevations in 3-MT are due to methylation of L-DOPA by catechol *O*-methyl-transferase. 3-MT has a long half-life in plasma (15 hours) and CSF; therefore, it is a key diagnostic marker for AADC deficiency.[3-6] Several pharmacologic approaches have been used and recommended for treatment; however, consistent therapeutic results have not been achieved across patient populations.[1] Targeted genetic therapies are also being explored and utilized to treat AADC deficiency.[4,7]

L-DOPA is produced from the amino acid tyrosine through the enzyme tyrosine hydroxylase. L-DOPA is also the precursor for the monoamine or catecholamine neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). Dopamine is formed by the decarboxylation of L-DOPA by AADC. L-DOPA can be directly metabolized by catechol-*O*-methyl transferase to 3-*O*-methyldopa and then further metabolized to vanillactic acid. In addition to patients diagnosed with AADC deficiency, L-DOPA metabolism is important for patients diagnosed with Parkinson disease—who are frequently treated with medications that raise L-DOPA levels.[8,9]

We recently reported an extreme elevation of 3-MT, detected by clinical global biochemical profiling of plasma, as a viable marker and less invasive method to identify AADC deficiency.[10] Untargeted mass spectrometry can identify compounds routinely tested in patients with inborn errors of metabolism, as well as analytes for which no clinical testing is available in the United States.[11,12] However, treatment with DOPA-raising medications also raises plasma 3-MT levels, which could limit the detection of AADC deficiency. Here, we describe a broader metabolic analysis of dopamine metabolites and demonstrate that the pattern of metabolite levels can be used to distinguish AADC deficiency from dopamine-raising therapies.

Materials and Methods

Sample collection

Specimens used in this analysis were referred to our clinical biochemical genetics laboratory for clinical metabolomic testing. All plasma samples were isolated from whole blood collected in ethylenediaminetetraacetic acid-containing tubes at the site of collection and frozen. Plasma samples were shipped frozen to our laboratory.

Patient presentations

Clinical information was submitted as part of sample submission requirements for clinical metabolomic testing. All procedures followed were in accordance with the ethical standards of the U.S. Department of Health and Human Services and were approved by the Baylor College of Medicine Institutional Review Board in accordance with the 1975 Declaration of Helsinki, as revised in 2000. This study was approved with a waiver of informed consent. Clinical data are summarized in Table 1.

Case 1

This child was previously reported by Atwal et al.[10] Briefly, the patient was initially evaluated for developmental delay at 11 months of age, with significant axial and appendicular hypotonia, speech delay, infrequent spells of generalized stiffening, facial hypotonia with bilateral ptosis, and a tented upper lip. Seizure-like episodes were noted and confirmed by electroencephalography, but the patient was lost to follow-up for ~2 years, when whole exome sequencing (WES) identified compound heterozygous missense variants in *DDC*. At four years of age, he exhibited frequent oculogyric crises, profound global hypotonia, with decreased muscle bulk, hyperreflexia, and static development.

Case 2

This is a girl of Taiwanese ancestry born at 38 weeks gestation to a nonconsanguineous 31-year-old G2P0>1 mother and 31-year-old father. There were no complications, illnesses, or exposures during the pregnancy. Shortly after birth, she was noted to have feeding difficulties and respiratory distress and spent 10 days in the neonatal intensive care unit. After discharge, it was noted that she had noisy breathing and would take extended amount of time to feed. She would also sweat during feeds and while asleep. She presented to the emergency center at our institution at six months of age with a history of increased congestion and difficulty breathing. She was also noted to have delays in fine motor and gross motor development, including hypotonia, feeding difficulties, and poor head control, as well as increased sweating and "breath holding spells." On examination, she had severe hypotonia with few spontaneous movements, decorticate posturing, microcephaly, and a sloping forehead. Her length, weight, and head circumference were at the 12th, 15th, and 2nd percentiles for age, respectively.

A swallow function study showed poor suck reflex with evidence of aspiration, leading to the commencement of nasogastric tube feedings. A brain MRI performed at 6.5 months of age showed borderline microcephaly, mild delays in myelination with a small, subcentimeter cystic-appearing nonenhancing lesion of uncertain etiology on the body of the right lateral ventricle. MR spectroscopy was normal. Electroencephalography was abnormal due to mild, diffuse slowing for age, consistent with the presence of a mild diffuse brain disturbance. No epileptiform activity was noted. Echocardiogram and ophthalmology evaluations were normal. Subsequent extensive laboratory profiling, including CSF glucose

TABLE 1.
Patient Demographics, *DDC* Variants, and DOPA Medications

Patient Number	Genetic Information	Diagnosis/Medication	Age	Sex
1	c.286G>A (p.G96R) and c.260C>T (p.P87L)	AADC deficiency, no L-DOPA meds	4 yr	M
2	c.714+4A>T (IVS6+4A>T) homozygous	AADC deficiency, no L-DOPA meds	9 mo	F
3	No *DDC* variants on WES	Carbidopa-L-DOPA	10 yr	M
4	No *DDC* variants on WES	Sinemet	2 yr	M
5	No *DDC* variants on WES	Sinemet	9 yr	M
6	No *DDC* variants on WES	Carbidopa/Banzel/Onfi	4 yr	F
7	No *DDC* variants on WES	Sinemet	4 yr	M

Abbreviation:
WES = Whole exome sequencing.