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Case Report

Newborn screening for dihydrolipoamide dehydrogenase deficiency: Citrulline as a useful analyte

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ABSTRACT

Dihydrolipoamide dehydrogenase deficiency, also known as maple syrup urine disease (MSUD) type III, is caused by the deficiency of the E3 subunit of branched chain alpha-ketoacid dehydrogenase (BCKDH), α-ketoglutarate dehydrogenase (αKGDH), and pyruvate dehydrogenase (PDH). DLD deficiency variably presents with either a severe neonatal encephalopathic phenotype or a primarily hepatic phenotype. As a variant form of MSUD, it is considered a core condition recommended for newborn screening. The detection of variant MSUD forms has proven difficult in the past with no asymptomatic DLD deficiency patients identified by current newborn screening strategies. Citrulline has recently been identified as an elevated dried blood spot (DBS) metabolite in symptomatic patients affected with DLD deficiency. Here we report the retrospective DBS analysis and second-tier allo-isoleucine testing of 2 DLD deficiency patients. We show that an elevated citrulline and an elevated allo-isoleucine on second-tier testing can be used to successfully detect DLD deficiency. We additionally recommend that DLD deficiency be included in the "citrullinemia/elevated citrulline" ACMG Act Sheet and Algorithm.

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1. Introduction

Dihydrolipoamide dehydrogenase (DLD) deficiency (OMIM #246900), also known as maple syrup urine disease (MSUD) type III, is an autosomal recessive disorder caused by deficiency of the E3 subunit of 3 mitochondrial enzyme complexes: branched chain alpha-ketoacid dehydrogenase (BCKDH), α-ketoglutarate dehydrogenase (αKGDH), and pyruvate dehydrogenase (PDH) [1]. DLD also functions as the L protein of the glycine cleavage systems [2]. The phenotype of DLD deficiency is variable, with patients presenting from the neonatal period to the 3rd decade of life with an early-onset neurologic phenotype or a primarily hepatic phenotype [3–5]. As one of the variant phenotypes of MSUD, DLD deficiency is a core condition recommended for newborn screening programs by the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) [6,7].

Newborn screening for MSUD in the US is performed by tandem mass spectrometry (MS/MS) measurement of dried blood spot (DBS) total leucine (Xle) [8]. Screen positive patients are identified through a combination of state-specific Xle cutoffs and ratios including Xle:Ala and/or Xle:Phe [9]. The detection of variant MSUD phenotypes using these strategies has been difficult, with multiple intermediate and intermittent phenotype patients escaping detection [10,11]. The addition of allo-isoleucine as a secondary analyte improves the detection of some variant forms of MSUD [11]. Of these previously reported patients with variant forms of MSUD, none were affected with DLD deficiency. Additionally, the use of Xle or allo-isoleucine in DLD deficiency may be less sensitive, as these patients can have normal branched-chain amino acid levels, even when acutely ill [12–14].

Citrulline has been recently identified as an elevated analyte in both plasma and on newborn DBSs in DLD deficiency [15]. For the first time we report the detailed retrospective NBS results, including second-tier allo-isoleucine testing, of 2 unrelated patients with DLD deficiency. One patient displayed an elevated citrulline, Xle and allo-isoleucine, the other showed an elevated Xle, Xle:Phe ratio and allo-isoleucine. Hypercitrullinemia, when combined with age-specific Xle and Xle:Phe measurements and second-tier allo-isoleucine testing, can be used to detect DLD deficiency in the newborn period. Additionally, we suggest that DLD deficiency be added to the elevated citrulline ACMG ACT sheet differential diagnosis and urine organic acid analysis be added to the elevated citrulline algorithm.

2. Materials and methods

2.1. Patients

Patients 1 and 2 were diagnosed clinically and not identified by Michigan's newborn screening program.

Patient 1 is a male infant born at term with a normal birth weight. Pregnancy was complicated by diet-controlled gestational diabetes. At 6 h of life, he presented with respiratory distress and hypoglycemia. He was admitted to the neonatal intensive care unit (NICU) at his local hospital where he spent one month for evaluation of hypoglycemia. NBS was completed at 24 h of life and 13 days of life (per hospital's NICU protocol) and both screens were reported as normal. At the time of discharge, his symptoms had resolved and were felt to be consistent with an infant of a diabetic mother.

Infancy was complicated by frequent emesis. At 7 months he presented to a local emergency department (ED) with lethargy, lactic acidosis and hypoglycemia. Plasma amino acids revealed a mildly elevated leucine at 181 μmol/L (48–175) and an allo-isoleucine level at 9 μmol/L (<2). At 8 months of age he presented again to the ED due to recurrent emesis. During that admission, laboratory values were normal besides urine organic acids showing a large peak of 2-oxoglutarate. *DLD* sequencing was completed based on the presence of plasma allo-isoleucine and elevated urine 2-oxoglutarate, suggesting deficiencies of both BCKDH and αKGDH, respectively (see below).

Patient 2 has been reported previously [5].

2.2. Patient samples

Patient 1's DBSs were collected at 24 h of life when he was symptomatic and at 13 days of life when he was clinically well but receiving total parenteral nutrition.

Patient 2's DBS was collected at 24 h of life when she was clinically well. This sample was initially analyzed prior to the incorporation of MS/MS into Michigan's screening laboratory and had been stored at room temperature in a humidity-uncontrolled room. Retrieval of the DBS and amino acid analysis by MS/MS and allo-isoleucine analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS) was performed and compared to 10 additional samples with similar birth dates and storage conditions as sample degradation was expected [16].

2.3. DBS analysis

DBSs were analyzed in Michigan's newborn screening laboratory using the Perkin Elmer NeoBase non-derivatized MSMS method. Michigan's age dependent cutoffs are detailed in Table 1.

2.4. Second-tier testing

Allo-isoleucine testing was performed at Mayo Medical Laboratories via LC–MS/MS.

3. Results

3.1. Patient 1

Detailed DBS results are listed in Table 1. At 24 h of life, Xle and Cit levels were elevated, with normal Xle:Phe, Xle:Ala, and Cit:Arg ratios. At 13 days of life Xle and Cit levels had normalized but with elevated Xle:Phe and Xle:Ala ratios. Retrospective second-tier testing showed mildly elevated allo-isoleucine levels of 5.5 μmol/L and 4.3 μmol/L (normal < 2.0) at 24 h and 13 days of life, respectively.

DLD sequencing identified compound heterozygosity for 2 pathogenic mutations inherited in *trans*: a c.1058 T > C (p.Ile353Thr) missense mutation and a novel 7-base pair microdeletion, c.1416_1422del ATATGGA (p.Tyr473HisfsStop6).

3.2. Patient 2

Retrieved DBS (after 17 years of storage) citrulline level was 6 μmol/L, not significantly different from similarly stored population controls ($p = 0.81$). Xle level was 122 μmol/L, significantly higher than population controls (control mean 40, standard deviation 5.6; Z-score: 14.5, $p < 0.0001$). Xle:Phe ratio was 4.4, significantly higher than population controls (control mean 2.2, standard deviation 0.39; Z-score: 5.9, $p < 0.0001$). Second-tier testing revealed an elevated allo-isoleucine level of 3.7 μmol/L, significantly higher than stored population controls (control mean 0.61, standard deviation 0.13; Z-score: 23.8, $p < 0.0001$).

Table 1

	Patient 1		MI state cutoffs		
	24 h (%ile)	13 days (312 h)(%ile)	≤72 h	>72 h & ≤180 h	>180 h
Xle (μmol/L)	*246* (*99.9*)	285 (96.2)	<175	<300	<350
Xle:Phe	2.9 (99.5)	*6.1* (*99.8*)	<4.0	<5.0	<5.5
Xle:Ala	0.65 (99.2)	*1.0* (*99.7*)	1.25	1.25	1.25
Cit (μmol/L)	*83* (*100.0*)	21 (51.6)	45	45	75
Cit:Arg	1.79 (58.0)	0.5 (9.1)	4.0	4.0	4.0
Allo-isoleucine (μmol/L)	*5.5*	*4.3*	–	–	–
Valine	172 (97.8)	215 (83.8)	340	340	340
Alanine	382 (86.7)	279 (20.4)	–	–	–

Newborn screening results of Patient 1 and Michigan cutoff levels. Bolded numbers represent abnormally elevated values. Note that the Xle:Ala ratio is not used in Michigan's screening logic. MSUD screening is the only condition in which 3 different age-specific analyte values are used. Normal allo-isoleucine <2 μmol/L. No cutoff has been determined for Alanine. Percentiles were calculated from non-NICU and non-TPN treated infants for each designated age range, between 1/31/2011 and 6/23/2014, $N_{Total} > 340{,}000$.

DLD sequencing identified compound heterozygosity for 2 pathogenic mutations inherited in *trans*: a c.405_407delAGG (p.G136del) truncating mutation and the c.1058 T > C (p.I353T) mutation also present in Patient 1.

4. Discussion

Newborn screening for dihydrolipoamide dehydrogenase, like other variant forms of MSUD, can be difficult [10,11]. It has been shown that citrulline levels are elevated in patients with DLD deficiency [15]. This prompted the retrospective investigation of 2 patients diagnosed clinically at our institution with DLD deficiency, not detected by Michigan's newborn screening program.

Patient 1 exhibited an interesting pattern of abnormalities including mildly elevated Xle and hypercitrullinemia at 24 h of life, with subsequent citrulline normalization and the development of an abnormal Xle:Phe ratio at 13 days of life when clinically well. Second-tier testing identified mild allo-isoleucine elevations in both samples. Patient 2's sample (retrieved and analyzed after 17 years of storage) exhibited a normal citrulline level but still displayed a significantly elevated Xle level and Xle:Phe ratio compared to normal controls stored in similar conditions. Second-tier testing also identified significantly elevated allo-isoleucine levels compared to population controls despite analysis after 17 years of storage. Patient 2 was clinically well at the time of sample collection.

All DLD patients with DBS hypercitrullinemia ($n = 3$) have been symptomatic at the time of collection, suggesting that citrulline elevations may only occur during times of metabolic decompensation [15]. Indeed, all previously reported DLD patients with plasma hypercitrullinemia, including both Patients 1 and 2 (data not shown), have only displayed plasma citrulline elevations during metabolic decompensations, with normalization when clinically well [5,18,19]. Although the mechanism for the accumulation of citrulline in DLD deficiency is unknown, Haviv et al. propose that it may be a result of insufficient oxaloacetate production, with secondary aspartate depletion and subsequent interference with argininosuccinate formation in the urea cycle [15]. DBS allo-isoleucine levels appear to be elevated regardless of clinical status as both Patients 1 and 2 displayed mild elevations. This further supports the importance of second-tier allo-isoleucine testing in detecting variant MSUD [11,17]. Given the small number of patients analyzed, further study is needed to confirm these results and determine the exact biochemical mechanism causing citrulline elevations.

Currently, the elevated citrulline ACMG ACT Sheet differential diagnosis includes citrullinemia I, argininosuccinic aciduria, citrullinemia II, and pyruvate carboxylase deficiency (https://www.acmg.net/StaticContent/ACT/Citrullinemia.pdf). The incidence of DLD deficiency in the Ashkenazi Jewish population is estimated to be 1:35,000, exceeding that of pyruvate carboxylase deficiency (1:125,000), argininosuccinic aciduria (1:70,000), and citrullinemia I (1:57,000) [20–22]. Patient 1's genotype also broadens the applicability to the US population, as it was previously suggested that only patients harboring the Ashkenazi Jewish p.G229C founder mutation presented with hypercitrullinemia [15].

In conclusion, we provide further evidence that DLD deficiency may be detected on newborn screening through the presence of an elevated citrulline and an elevated allo-isoleucine on second-tier testing. Based on this, in the setting of an elevated citrulline, especially with abnormal Xle or Xle:Phe ratio, the addition of allo-isoleucine testing into the screening algorithm may improve the diagnosis of DLD deficiency by NBS. Though further study is needed to investigate citrulline's utility in screening for DLD deficiency, its use as described here will not significantly increase false positive cases and has the potential to identify DLD deficiency patients who may have been previously undiagnosed by current screening logic. We recommend that DLD deficiency be included in the Citrullinemia ACMG Act Sheet differential diagnosis and that the algorithm include urine organic acid analysis.

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