Thiamine-Responsive and Non-responsive Patients with PDHC-E1 Deficiency: A Retrospective Assessment

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Abstract Pyruvate dehydrogenase complex (PDHC) deficiency is a disorder of energy metabolism that leads to a range of clinical manifestations. We sought to characterise clinical manifestations and biochemical, neuroimaging and molecular findings in thiamine-responsive and nonresponsive PDHC-deficient patients and to identify potential pitfalls in the diagnosis of PDHC deficiency. We retrospectively reviewed all medical records of all PDHC-deficient patients ($n = 19$; all had *PDHA1* gene mutations) and one patient with severe PDHC deficiency secondary to 3-hydroxyisobutyryl-CoA hydrolase deficiency managed at our centre between 1982 and 2012. Responsiveness to thiamine was based on clinical parameters. Seventeen patients received thiamine treatment: eight did not respond, four showed sustained response and the others responded temporarily/questionably. Sustained response was noted at thiamine doses >400 mg/day. Age at presentation was 0–6 and 12–27 months in the nonresponsive ($n = 8$) and responsive

($n = 4$) patients, respectively. Corpus callosum abnormalities were noted in 4/8 nonresponsive patients. Basal ganglia involvement (consistent with Leigh disease) was found in four patients (including 2/4 thiamine-responsive patients). Diagnosis through mutation analysis was more sensitive and specific than through enzymatic analysis. We conclude that patients presenting at age >12 months with relapsing ataxia and possibly Leigh syndrome are more likely to be thiamine responsive than those presenting with neonatal lactic acidosis and corpus callosum abnormalities. However, this distinction is equivocal and treatment with thiamine (>400 mg/day) should be commenced on all patients suspected of having PDHC deficiency. Mutation analysis is the preferable first-line diagnostic test to avoid missing thiamine-responsive patients and misdiagnosing patients with secondary PDHC deficiency.

Short Summary: Thiamine responsiveness is more likely in patients presenting at age >12 months with relapsing ataxia and possibly Leigh syndrome than in those presenting with neonatal lactic acidosis and corpus callosum abnormalities. Thiamine doses >400 mg/day are required for sustained response. Mutation analysis is more sensitive and specific than enzymatic analysis as a first-line diagnostic test.

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Abbreviations

HIBCH	3-Hydroxyisobutyryl-CoA hydrolase
MRI	Magnetic resonance imaging
PDHC	Pyruvate dehydrogenase complex
TPP	Thiamine pyrophosphate

Introduction

The pyruvate dehydrogenase complex (PDHC) is pivotal for energy metabolism in that it catalyses the oxidative decarbo-

xylation of pyruvate into acetyl-CoA, linking glycolysis to the tricarboxylic acid cycle. The PDHC is comprised of several copies of enzymatic subunits, E1 (pyruvate dehydrogenase), E2 (dihydrolipoamide transacetylase) and E3 (dihydrolipoamide dehydrogenase) and an E3 binding protein (E3BP), and its activity is regulated by several isoforms of kinases and phosphatases through reversible phosphorylation (Linn et al. 1969; Pagliarini and Dixon 2006). The E1 enzyme consists of two α - and two β -subunits that share a binding site for thiamine pyrophosphate (TPP), a metabolite of thiamine, which is an essential cofactor for the enzymatic reaction and also helps to maintain the PDHC in an activated state by inhibiting its phosphorylation (Roche and Reed 1972).

PDHC deficiency causes impairment of energy metabolism and leads to a broad range of symptoms. Four main neurological presentations have been reported: neonatal encephalopathy with lactic acidosis, non-progressive infantile encephalopathy, Leigh syndrome and relapsing ataxia (Robinson et al. 1987; Brown et al. 1988, 1989a; Barnerias et al. 2010; Patel et al. 2012). The majority of patients have a mutation located in the *PDHA1* gene encoding the E1 α subunit, which is located on the X chromosome (Robinson and Sherwood 1984; McKay et al. 1986; Wicking et al. 1986; Brown et al. 1989b; Lissens et al. 2000). The differences in presentation result from variations in mutations and from the degree of X inactivation in females (Brown et al. 1989b; Dahl et al. 1992).

A high-fat diet, with the amount of calories provided from fat exceeding 50%, has been shown to be effective in many patients with PDHC deficiency. It leads to decreased blood lactate and pyruvate concentrations and provides an alternative source of energy in the form of ketones (Wexler et al. 1997) and has been shown to be helpful in reducing childhood onset epilepsy and paroxysmal dystonia (Barnerias et al. 2010). However, its efficacy has been variable (Weber et al. 2001). The administration of thiamine is an additional treatment that can potentially be effective, given its role as a cofactor (as TPP) of the enzyme-complex activity (Di Rocco et al. 2000; Lee et al. 2006; Barnerias et al. 2010; Giribaldi et al. 2012).

The diagnostic process usually includes measuring PDHC activity in cultured fibroblasts or skeletal muscle, but there may be great variation in fibroblast activities reported by different laboratories. Thus, it is likely that not all patients are correctly diagnosed and therefore the exact prevalence of PDHC deficiency is not known (Barnerias et al. 2010; Patel et al. 2012). Moreover, thiamine-responsive patients might be missed, because enzyme analysis is usually performed with high TPP concentrations and will not show a decreased PDHC enzyme activity in these patients (Di Rocco et al. 2000; Naito et al. 2002a; Lee et al. 2006). Amongst patients with thiamine-responsive

PDHC deficiency, several different mutations have been found; the majority located in the *PDHA1* gene, encoding the E1 α subunit, some in the region encoding the TPP-binding site, some outside the TPP-binding site (Naito et al. 1994, 2002a, b; Benelli et al. 2002; Debray et al. 2008). At present, it is not known whether thiamine-responsive patients present differently from those not responsive to thiamine and what doses of the vitamin are required to gain significant therapeutic benefits.

The aim of this study was to review all clinical and laboratory data on our patients with PDHC deficiency in order to elucidate whether a prediction of thiamine responsiveness can be made in patients with PDHC deficiency based on clinical, biochemical or neuroimaging studies and to demonstrate the difficulties in diagnosing PDHC deficiency accurately and reliably by enzymatic analysis. To this end, we included in this study a patient with secondary PDHC deficiency, presumed to have primary PDHC deficiency at the time of presentation.

Methods

We conducted a retrospective review of all medical records of all patients with symptoms and biochemical findings consistent with PDHC deficiency at the Royal Children's Hospital in Melbourne, Australia. Included patients were born in Victoria or Tasmania between 1 January 1982 and 2012. Available clinical, laboratory and neuroimaging characteristics were collected into a database. We defined patients with PDHC deficiency as those with a pathogenic mutation in one of the genes encoding the PDHC subunits or a combination of clinical presentation consistent with PDHC deficiency (e.g. neonatal lactic acidosis, Leigh disease, relapsing ataxia etc.) and diagnostically low PDHC enzyme activity with no other confirmed diagnosis. We also included in this study one patient with severe PDHC deficiency secondary to 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency determined enzymatically. The study was approved by the Human Research Ethics Committee of the Royal Children's Hospital (HREC # 32087A).

Enzyme analyses were performed usually in cultured skin fibroblasts as previously published (Rahman et al. 1996). Briefly, total (dichloroacetate-activated) PDHC activity was assayed by measuring the rate of ^{14}C -CO₂ formation from [1- ^{14}C]pyruvate. PDHC activity was also measured in lymphocytes and/or skeletal muscle biopsies from some patients. Activities were expressed as percentages of normal control mean relative to protein concentration and relative to citrate synthase. Mutations were identified by sequencing the coding region of the *PDHA1* gene in cDNA prepared from patient fibroblasts and then confirmed in genomic DNA as described previously (Ridout et al. 2008).

Clinical characteristics included pregnancy and perinatal history, age at onset, presenting symptoms and signs, details on thiamine treatment and outcome. Responsiveness to thiamine treatment was defined as sustained clinical improvement (i.e. regaining spontaneous breathing after a period of intubation; decline in seizure activity; developmental gain with progress in communication, intellectual or motor skills; improvement in mobility, tone or coordination; or a decrease in movement disorders (Pastoris et al. 1996; Di Rocco et al. 2000; Naito et al. 2002a, b)) and/or improvement suggested by neuroimaging after the start of thiamine administration. Findings of brain computerised tomography (CT), ultrasonography (US) or magnetic resonance imaging (MRI) before and after the start of thiamine treatment were reviewed, as available. The lowest and highest concentrations of lactate, pyruvate and alanine in blood (arterial, venous and capillary) and cerebrospinal fluid (CSF) before and after start of thiamine treatment were noted but are not reported here. We did not consider biochemical improvement in the definition of responsiveness to thiamine because values of lactate and pyruvate range broadly, are inconsistent and can be influenced by a variety of factors, as has been well documented previously (Chariot et al. 1994). Moreover, lack of correlation between lactate and pyruvate concentrations and clinical outcome has been shown in previous studies on treatment of patients with PDHC deficiency (Barnerias et al. 2010).

Results

We identified 19 patients with primary PDHC deficiency and one patient with secondary PDHC deficiency due to 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency. Details regarding the presenting symptoms and signs and confirmation of diagnosis are presented in Table 1. In the group of patients with PDHC deficiency feeding difficulties were a common presenting symptom in the neonatal period ($n = 7/9$) as well as lethargy (5/9), hypotonia (4/9) and abnormal respiration (3/9). Seizures were noted in two patients who presented in the neonatal period and two who presented later. Intermittent ataxia was noted in two patients (or possibly three, including patient 15). The patient with HIBCH deficiency also presented with feeding difficulties.

Other neurological and non-neurological symptoms and signs, noted through follow-up reviews, are summarised in Table 2. Ataxia was noted in 8/19 and dystonia in 4/19 patients, combined with chorea in two. Tremor was noted in 3/19 patients. Microcephaly was documented in 4/19 patients, all female. One female patient (patient 19, sister of patient 18) was asymptomatic. Only 4/19 patients had normal development.

Details on treatment and responsiveness to treatment are summarised in Table 3. High-fat diet (50–60% of total calorie intake) was prescribed routinely or as part of a “sick day regime” in 15 patients, alone or in combination with thiamine. Two patients (patients 2 and 5) did not receive thiamine treatment and one was lost to follow-up (patient 14). Eight patients (patients 3, 4, 6, 7, 9, 10, 12, 13) received thiamine at doses ranging from 50 mg to 300 mg/day with no clinical effect. All were on a high-fat diet (including one on expressed breast milk) and five were on anticonvulsants. The age at onset in these patients ranged from the neonatal period (five patients) to 6 months. Seizures occurred in 6/8, dystonia and bulbar dysfunction were noted in 3/8 and chorea in 2/8 patients. Ataxia was reported in only 2/8 patients. Five of these patients have deceased. Patients 1 and 11 (age at presentation: neonatal and 2 months, respectively) seemed to respond favourably but temporarily and deceased later (one at age 1 year; ~25 years ago). Both had seizures and one had chorea.

Four patients (patients 15, 16, 17, 18) were treated with thiamine doses 200–1,200 mg/day and showed sustained response at doses above 500 mg/day. Of these, one was not on a high-fat diet, another was only temporarily on the diet and one was on a “moderately” (40%) high-fat diet. The age at presentation of these patients ranged from 12 to 27 months. Ataxia was reported in 3/4, tremor in 2/4 and bulbar dysfunction and chorea/dystonia in one patient, each. Seizures occurred in only one of these patients. Two of the three patients who regained spontaneous breathing after a period of acute deterioration had only transient response; hence, this feature cannot be ascribed unequivocally to thiamine responsiveness and may reflect the natural history of the disorder. Patient 8, who presented in the neonatal period, is severely disabled, and although she seems to have improved on thiamine treatment, it is hard to confirm improvement objectively. The patient with HIBCH deficiency did not respond to thiamine.

Neuroimaging was done in all 20 patients except for one nonresponsive patient and one asymptomatic PDHC-deficient patient (Table 4). Structural abnormality of the corpus callosum was noted in 6/19 patients (4/9 patients who presented in the neonatal period; two who presented in the second month of life, including one prenatal ultrasonographic diagnosis). Of these patients, 4/6 did not respond to thiamine treatment, 1/6 responded temporarily and 1/6 did not receive thiamine treatment. Cystic lesions were noted in 6/9 patients who presented in the neonatal period. Pre-thiamine treatment imaging revealed basal ganglia involvement (consistent with Leigh disease) in 2/4 thiamine-responsive patients, one who was not treated with thiamine and one patient who had transient response to treatment. The patient with HIBCH deficiency had “abnormalities in brainstem and basal ganglia”.

Table 1 Presenting symptoms/signs and results of confirmatory tests

Patient number/sex	Age at onset	Presenting symptoms/signs	PDHC activity (%)	Mutation
1/male	Neonatal	Feeding difficulties Lethargy Irregular breathing Hypertonia	F: 39	PDHA1: c.1133G > A, p.Arg378His
2/female	Neonatal	Feeding difficulties Lethargy Hypotonia	F: 36 (PDH E1: 28) SM: 39	PDHA1: c.419-(17_14)delTGTT
3/female	Neonatal	Feeding difficulties Lethargy Hypotonia Seizures	F: 21	PDHA1: c.927_933delAGTAAGA, p.Ser312Valfs11
4/male	Neonatal	Facial asymmetry Feeding difficulties Lethargy Respiratory distress	F: 32	PDHA1: c.483C > T, p.Tyr161Tyr
5/male	Neonatal	Hypotonia Seizures Feeding difficulties Lethargy Respiratory distress	F: 35	PDHA1: c.604-10C > G
6/female	Neonatal	Hypotonia Feeding difficulties	F: 19	PDHA1: c.511G > A, p.Val171Met
7/female	Neonatal	Feeding difficulties Hyperextension neck	F: 50	PDHA1: c.947C > T, p.Pro316Leu
8/female	Neonatal	Mild lethargy	F: 33 (relative to protein)	PDHA1: c.1139_1142dupCCAA, p.Trp383Serfs5
9/female	Neonatal	Congenital retinal dystrophy	F: 40 (PDH E1: 37)	PDHA1: c.853C > T, p.Gln285*
10/male	M2	Seizures Respiratory distress	F: 43 L: 53	PDHA1: c.628A > G, p.Met210Val
11/male	M2	Episodes of irregular breathing Seizures	F: 11	PDHA1: c.1132C > T, p.Arg378Cys
12/male	M4	Feeding difficulties Hypotonia	F: 43 L: 18 SM: <5	PDHA1: c.1156_7insT, p.Lys387*
13/female	M6	Feeding difficulties Lethargy Hypotonia Developmental delay	L: 39	PDHA1 c.1156_7insT, p.Lys387*

14/female	M12	Developmental delay Progressive microcephaly	F: 64	PDHA1: c.858_861dupTTAC, p.Arg288Leufs9
15/male	M12	Tremor Frequent falling General weakness	F: 37	PDHA1: c.787C > G, p.Arg263Gly
16/male	M14	Intermittent ataxia	F: 75	PDHA1: c.787C > G, p.Arg263Gly
17/female	M24	Developmental delay Right hemiparesis	F: 10	PDHA1: c.1156_7insT, p.Lys387*
18/male	Y2M3	Intermittent ataxia	F: 41	PDHA1: c.261T > G, p.Ile87Met
19 ^a /female	(Cascade testing)	No symptoms	ND	PDHA1: c.261T > G, p.Ile87Met
20 ^b /female	Neonatal	Feeding difficulties	F: 26	

Y years, *M* months, *Sz* seizures, *F* fibroblasts, *L* lymphocytes, *SM* skeletal muscle

^a Asymptomatic female patient diagnosed through cascade testing

^b PDHC deficiency secondary to HIBCH deficiency

Table 2 Follow-up: neurological and non-neurological manifestations

Patient/sex	Seizures (age)	Ataxia (age)	Movement disorder (age)	Tone/tendon reflexes	Other neurological signs	Other neurological symptoms and signs	Ophthalmological findings	Other
1/male	M1	–	–	Axial hypotonia, distal hypertonia/hyperreflexia	Aspiration			Episodes of cyanosis
2/female	–	–	–	Axial hypertonia, distal hypertonia				
3/female	D6	–	–	Axial hypotonia, fluctuating distal tone	–			
4/male	D1	–	Dystonia (D13)	Axial hypotonia, distal hypertonia	Autonomic dysfunction			
5/male	M19	–	–	Generalised hypotonia/hyperreflexia	Bulbar dysfunction	Fluctuating ptosis		
6/female	M4	M14	Chorea/dystonia (Y9M10)	Axial hypotonia, distal hypertonia	Microcephaly	Alternating exotropia (Lt)	Skeletal deformities	
					Dysphagia	Pale optic disks	Fatigue	
7/female	–	–	–	Spastic tetraparesis/hyperreflexia	Microcephaly		Peripheral oedema	
					Cortical blindness		Skeletal deformities	
8/female	–	Y5M2	–	Axial hypotonia, distal hypertonia/hyperreflexia	Microcephaly	Ptosis (Lt)	Irritability hepatomegaly	
					Mild pseudobulbar dysfunction	Retinal changes	Skeletal deformities	
					Sensorineural hearing loss			
9/female	Y3	–	–	Axial hypotonia, distal hypertonia/hyperreflexia	Microcephaly	Divergent squint	Hyperlaxity of small joints	
					Dysphagia		Sleep disturbance	
					Lethargy			
					Contractures			
10/male	M2	–	Intermittent dystonia (Y10)	Axial hypotonia	Lethargy	Episode of unequal pupils	Feeding difficulties	
				Developed spastic tetraparesis/hyperreflexia	Axonal neuropathy		Skeletal deformities	
					Aspiration		Growth retardation	
					Episodes of respiratory distress		Anxiety/panic attacks	
							Easy bruising	
11/male	M2	–	Chorea (M6)	Axial hypotonia, distal hypertonia	Lethargy	Pale optic disks; nystagmus	Feeding difficulties	
					Distal weakness		Skeletal deformities	
					Bulbar dysfunction			
					Episodes of irregular breathing			
					Fluctuating consciousness			

12/male	–	–	–	Generalised hypotonia	Episodes lethargy and sweating Quadriplegia Bulbar dysfunction Respiratory decompensation Episodes of irregular breathing Contractures	Alternating divergent squint Pale optic disks	Skeletal deformities Osteopenia Patella alta Premature pubarche Prolonged QT interval Fatigue Headaches Mild memory loss Episodic chest pain Sleep disturbance Growth retardation Autistic features
13/female	M22	Y3M9	Tremor (Y2M1)	Axial hypotonia, distal hypertonia			
14/female	–	Y4M11	–	Spastic tetraparesis	–		
15/male	–	M12	Tremor (M12)	Generalised hypotonia/areflexia	Lethargy Episodic respiratory decompensation Bulbar dysfunction Fasciculations Dysphagia Respiratory decompensation Dysarthria Sixth nerve palsy	Intermittent ophthalmoplegia Convergent strabismus	Feeding difficulties Fatigue Hypertension Tachycardia Prolonged QT interval orthopnoea Thumb hypermobility Episodes of hyperacusis Skeletal deformities Osteopenia Fatigue Headaches Short concentration span
16/male	M20	M14	Tremor (M20)	Generalised hypotonia/areflexia			
17/female	–	Y8M0	–	Distal hypertonia	Intermittent hemiplegia Dysidiadochokinesia later persistent hemiplegia Sensorineural hearing loss		

(continued)

Table 2 (continued)

Patient/sex	Seizures (age)	Ataxia (age)	Movement disorder (age)	Tone/tendon reflexes	Other neurological symptoms and signs	Ophthalmological findings	Other
18/male	–	Y2M3	Tics/grunts/chorea (Y3M1) Dystonia (Y5M0)	Episodic axial hypotonia Distal hypertonia	Hyperreflexia Episodic lethargy/ weakness Peripheral neuropathy Acute hemiparesis Dysarthria	Astigmatism	Skeletal deformities Growth retardation Headaches Daytime urine incontinence
19 ^a /female	–	–	–	Normal	–		
20 ^b /female	M3	–	–	Distal hypotonia Fluctuating distal tone	Episodic respiratory distress Aspiration	Optic atrophy	

Y years, M months, D days, Sz seizures

^a Asymptomatic female patient diagnosed through cascade testing

^b PDHC deficiency secondary to HIBCH deficiency

Enzyme analysis was done in all but patient 19, who was diagnosed by mutation analysis through cascade screening. Fibroblast PDHC activity, expressed relative to citrate synthase, ranged from 19% to 50% and from 10% to 75% of normal in thiamine-nonresponsive and thiamine-responsive patients, respectively (with no substantial difference between samples from male or female patients in these groups). In two thiamine-responsive patients (patients 14 and 16), enzyme activity was not low enough to be diagnostic for PDHC deficiency, and mutation analysis eventually established the diagnosis. Of particular note is the very low PDHC activity (26% of normal) in cultured fibroblasts of the patient later found to have HIBCH deficiency.

Pathogenic mutations leading to PDHC deficiency were identified in all patients with PDHC deficiency (Table 1). All our patients had mutations in the *PDHA1* gene (Table 1) with at least 7/19 having de novo mutations (three mothers were carriers; information regarding the other mothers is not available). Patients 1, 3, 4, 7, 10, 11, 15 and 16 had mutations that had been described previously. All other patients were found to have novel mutations, splicing, insertions and duplications (some in the same region as other similar mutations; e.g. p.Ile87Met in thiamine-responsive patients 18, 19). The patient with HIBCH deficiency was found to be a compound heterozygote for mutations in the HIBCH gene.

Discussion

We sought to identify clinical, laboratory and imaging findings that may predict clinical responsiveness to thiamine treatment in patients with PDHC deficiency. Despite the limitations of it being a retrospective observational study, with a limited number of patients, our results highlight the pitfalls in the clinical and biochemical diagnosis of PDHC deficiency and the need for high thiamine doses to achieve sustained clinical benefit from this treatment. Prospective studies with larger cohorts of patients are needed to further elucidate the clinical features that may clearly distinguish and enable prediction of responsiveness and the thiamine doses for sustained response.

The spectrum of clinical manifestations in our cohort of patients is in line with previous reports (Barnerias et al. 2010; Giribaldi et al. 2012; Patel et al. 2012). The high prevalence of feeding difficulties and lethargy soon after birth and the high prevalence of skeletal deformities are intriguing, as they have not been previously reported (Barnerias et al. 2010; Patel et al. 2012), possibly because they had not been directly associated with this diagnosis.

It is also likely that some of the skeletal deformities were secondary to dystonia. Our observations indicate that patients who present with relapsing ataxia and those with Leigh syndrome are more likely to be responsive to thiamine therapy. Ataxia has been previously described in thiamine-responsive patients (Kinoshita et al. 1997; Di Rocco et al. 2000) and in patients with mutations within the thiamine pyrophosphate domain (Debray et al. 2008). From a neuroimaging perspective, basal ganglia abnormalities were more common in thiamine-responsive patients, in support of the diagnosis of Leigh disease, whereas corpus callosum abnormalities and cystic lesions were found mainly in nonresponsive patients, in support of an early, possibly intrauterine insult to the CNS. However, these characteristics are not absolutely distinctive as they can be present in both responsive and nonresponsive patients. No differences were found in other clinical or biochemical characteristics (plasma and CSF lactate and pyruvate concentrations, PDHC enzyme activity).

Our observations highlight difficulties and potential pitfalls in diagnosing PDHC deficiency. For example, enzyme analysis of patient 16 was not low enough to be diagnostic for PDHC deficiency and a diagnosis could only be made through mutation analysis. Patient 18 was found to have a novel missense mutation adjacent to two previously reported missense mutations associated with thiamine responsiveness. This patient would not have been diagnosed by standard enzymatic assay as the activity in his cultured fibroblasts was borderline low yet within the normal range both with and without TPP in the assay mixture. Similarly, patient 12 had substantial residual PDHC activity in cultured cells but undetectable activity in skeletal muscle, possibly due to different levels of TPP in tissue versus culture media or to other factors affecting PDHC stability in different cell types. Such pitfalls have been previously described (Di Rocco et al. 2000; Lee et al. 2006). Interpretation of results is further complicated in female patients, where enzyme activity reflects the impact of X inactivation (e.g. patient 14). On the other hand, PDHC activity was very low in fibroblasts from the patient with HIBCH deficiency, who presented with a clinical phenotype, blood and CSF biochemistry and neuroimaging findings suggestive of PDHC deficiency, indicating that secondary PDHC deficiency could mistakenly lead to a diagnosis of PDHC deficiency unless molecular analysis is done. It may therefore be concluded that in order to avoid missing thiamine-responsive PDHC deficiency and misdiagnosing secondary PDHC deficiency, mutation analysis would be the preferred diagnostic test. Given that the vast majority of patients with PDHC deficiency have defects in the X-linked *PDHA1* gene, it would seem prudent to sequence that gene first.

Table 3 Treatment and response

Patient number/sex	Start/dose/day	Response	High-fat diet start	Anticonvulsants	Outcome (age)
1/male	D14/400 mg	Regained spontaneous breathing	D16	+	Deceased (Y1)
2/female	None	N/A	None	None	Deceased (M1D3)
3/female	D5/50 mg	No clinical effect	D7	+	Deceased (D27)
4/male	D21/150 mg	No clinical effect	Expressed breast milk	+	Deceased (M1D13)
5/male	None	N/A	None	+	Deceased (Y1M9)
6/female	Y9M10/150–300 mg	No clinical effect	Y10M8	+	Alive (Y13M6)
7/female	M9D19/NI	No clinical effect	Y1 (for 1 year)	None	Deceased (Y27M8)
8/female	M7D21/200–600 mg	Improved development	M9D17	None	Alive (Y7M6)
9/female	Y6M7/300 mg	No clinical effect	Y2M10D12 (stopped by family)	+	Alive (Y17M11)
10/male	M3D5/300 mg	No clinical effect	M3	+	Deceased (Y11M3)
11/male	M6D20/150–600 mg (1,200 mg attempted before death)	Regained spontaneous breathing Improved mobility and development	M14D20	+	Deceased (Y2M9)
12/male	M6D30/150 mg	Decline in seizures	M6	None	Deceased (Y1M1)
13/female	Y14M8/100 mg	No clinical effect	M6	None	Alive (Y18M4)
14/female	Y4M11/300 mg	No information	Y4M11		Lost to follow-up (Y6M6)
15/male	Y1M3/200–600 mg	Regained spontaneous breathing Improved mobility and strength	None	None	Alive (Y3M10)
16/male	Y1M8/300–1,200 mg	Resumed gait and speech within weeks. Attends regular kinder Improved coordination and tone Developmental gains: intellectual, communication and mobility	Y1M8 (for 1 year; then when unwell)	None	Alive (Y8M11)
17/female	Y13M6/100–500 mg	Improved neuropsychological performance at age Y7M3. Attends mainstream school Improved mobility and strength Increased energy level	Y16M3 (40% of calories)	None	Alive (Y31M4)
18/male	Y2M5/300–600 mg	Improved development: intellectual, communication and mobility (limited by contractures) Increased energy level	Y2M5	None	Alive (Y9M8)
19 ^a /female	Y3M5/300–600 mg	Remains asymptomatic	None	None	Alive (Y9M8)
20 ^b /female	Y5M2/200–300 mg	No clinical effect	None	+	Deceased (Y8M1)

Y years, M months, D days, Sz seizures

^a Asymptomatic female patient diagnosed through cascade testing^b PDHC deficiency secondary to HIBCH deficiency

Table 4 Neuroimaging

Patient number	Neuroimaging	
	Before thiamine Rx	After thiamine Rx
1	US: multiple cystic lesions in anterior aspect choroid plexus. Mild asymmetry and dilatation of ventricular system	CT (8 D after start Rx): no abnormalities US (14 D later): cystic lesions floor lateral ventricles, possible resolving sub-ependymal haemorrhage
2	US: CC agenesis, right choroid cyst. Gross dilatation lateral ventricles. Diminished amount of cerebral substance. Prominent cisterna magna	–
3	US: possible cystic structures floor lateral ventricles. Gross hydrocephalus lateral and third ventricles	–
4	MRI: thin CC, periventricular cysts, mild lateral and third ventriculomegaly. Immature cerebral sulcation. Under opercularisation. Globally abnormal signal white matter, generous extra-axial surface spaces. MRS: No elevated lactate	–
5	MRI: symmetric signal abnormalities cerebellar hemispheres, brain stem, R occipital pole, L hypothalamus (consistent with Leigh syndrome) MRI (15D later): interval evolution of signal abnormalities. Resolution of acute oedema, generalised cerebral atrophy. Possible signal changes ganglio-thalamic regions MRS: elevated choline, lactate and pyruvate	–
6	MRI: microcephaly, partial CC agenesis, posterior fossa arachnoid cyst. Asymmetrical ventriculomegaly. Cerebral atrophy Lt > Rt MRI (9Y10M later): myelination of remaining white matter occurred, no other changes MRS: abnormal lactate peaks over left periventricular white matter	–
7	–	–
8	MRI: asymmetric lateral ventricles, white matter loss Lt > Rt, cerebral atrophy. Extra-axial collection R frontal region	–
9	MRI: partial CC agenesis. Probable large arachnoid cyst posterior fossa. Possible stenosis in aqueduct of Sylvius causing hydrocephalus	–
10	Prenatal US: agenesis of the CC	MRI (1 M after start Rx): CC agenesis, dysmorphic ventricles. MRS: increase lactate MRI (5 M after stop Rx): CC agenesis, colpocephaly, signal change in globus pallidus and deep cerebellar white matter. MRS: lactate peak brainstem and basal ganglia CT(7 M after start Rx): stable prominent ventricles and extra-axial spaces
11	CT ^c : mild prominence lateral and third ventricles MRI: thin CC. Symmetrical signal abnormalities hippocampi, globus pallidi, lateral thalami (consistent with Leigh Syndrome). Enlarged extra-axial CSF spaces	–
12	–	–
13	MRI: brachycephaly. Possible white matter loss, but myelination not yet completed	–
14	CT: ventricular dilatation and cerebral atrophy	–
	MRI: cerebral atrophy	–
15	MRI: no abnormalities	–

(continued)

Table 4 (continued)

Patient number	Neuroimaging	
	Before thiamine Rx	After thiamine Rx
16	MRI: symmetrical signal changes in globus pallidi, putamen, dentate nuclei, ventral medulla and around fourth ventricle (consistent with Leigh syndrome) MRS: raised lactate	MRI ^d : decrease of signal changes except for increased signal in dentate nucleus. New areas in cerebral peduncles MRI (1 Y9 M after restart Rx): decrease in signal changes, areas partially replaced by more cystic changes MRI (5 M later): progression of swelling, patchy signal changes globus pallidi. Slight increase signal change medulla MRI (4 Y7 M later): Slight increase hyperintensity dorsal medulla. Decrease in signal changes and resolution of swelling, with residual cyst
17	MRI: decreased signal in L globus pallidus and small area of increased signal in R globus pallidus and signal change L cerebral peduncle MRI: no abnormalities	–
18		MRI (2 Y6 M after start Rx): extensive gliosis, bilateral cystic and asymmetrical signal changes basal ganglia MRI (6 M later): stable appearance
19 ^a	–	–
20 ^b	MRI: thin CC, prominence of ventricular system, cerebral atrophy. Delayed myelination, abnormalities brain stem and basal ganglia	–

Y years, M months, D days, CC corpus callosum, Lt left, Rt right

^a Asymptomatic female patient diagnosed through cascade testing

^b PDHC deficiency secondary to HIBCH deficiency

^c 4 days after starting thiamine treatment

^d 5 days after restarting thiamine treatment, after ceasing treatment for 6 months

It is difficult to establish the exact relationship between mutations in the *PDHAI* gene and “thiamine responsiveness”. Before the structure of the E1 enzyme was elucidated, a number of mutations were proposed to involve amino acid residues located within the TPP-binding region. These assignments are not consistent with the known structure of the enzyme, so interpretations of their biochemical consequences are difficult to evaluate. None of the mutations that have been well defined as causing “thiamine-responsive” PDHC deficiency have involved amino acid residues that are directly involved in TPP binding; most are adjacent to the binding site and probably alter the position of the actual side chains that interact with the cofactor, weakening their binding. There are two groups of these: p. Ile87Met (present report), p.Arg88Ser (Marsac et al. 1997), p.Arg88Cys (Fujii et al. 2006) and p.Gly89Ser (Naito et al. 1999) on one side and p.Phe205Leu (Naito et al. 2002a), p.Met210Val (Tripatara et al. 1999), p.Trp214 (Lissens et al. 2000), p.Leu216Phe (Naito et al. 2002a) and p. Pro217Leu (also denoted as Pro188Leu) (Hemalatha et al. 1995) on the other. A number of patients have been reported as thiamine responsive but have mutations involving amino acid residues that are located well away from the TPP-binding site. These include p.Val71Ala and p.Cys101Phe (Naito et al. 2002b), p.Tyr161Cys (Lee et al. 2006), p.Tyr243Ser (Benelli et al. 2002), p.Arg263Gly (Bachmann-Gagescu et al. 2009) and other C-terminal residues (Narisawa et al. 1992). At present, there is no structural explanation as to why these should influence TPP binding.

As the TPP-binding site is in the interface between the E1 α and β subunits, it might be expected that defects in either one could be thiamine responsive. However, given that the total number of E1 β patients is very small, it is not surprising that no such patients have been described so far. Patients with defects in thiamine transport or the activation of thiamine to TPP would also be expected to be thiamine responsive.

The proportion of thiamine-responsive patients in our cohort (4–5/19) is high in comparison with previous reports (Barnerias et al. 2010; Patel et al. 2012). This difference could be due to differing definitions of responsiveness, namely clinical, biochemical or other. In this regard, we found that clinical response does not correlate with biochemical response (e.g. lactate, pyruvate; data not shown). Another possible reason for our relatively high number of thiamine-responsive patients is an increasing awareness to the possibility of thiamine-responsive PDHC deficiency, coupled with molecular testing. Indeed, there has been an increase in our diagnosis of thiamine-responsive patients within the last 5 years. A third possibility is the use of high (>400 mg/day) or very high (>1,000 mg/day) thiamine doses in our patients. Previous

reports have highlighted the discrepancy between in vitro and in vivo response to thiamine. In some reports, recovery of activity has been demonstrated in vitro when the enzyme was assayed in high concentrations of TPP, but there has been no clear clinical response to vitamin supplementation. Debray et al. reported two siblings who were found to have a mutation in the thiamine-binding domain but did not show any clinical benefit from thiamine treatment, at 150 and 250 mg/daily, respectively (Debray et al. 2008). Likewise, Barnerias et al. reported only 1 of 22 patients who was thiamine responsive, but the daily thiamine dose was low (50 mg) (Barnerias et al. 2010). Patel et al. reviewed data on 371 patients and reported that only 73 received thiamine (from a few milligrams to >1,000 mg daily), but no information was given regarding the correlation between doses and responsiveness (Patel et al. 2012). Thus, there are insufficient reported data on the optimal daily thiamine dose that patients with PDHC deficiency should receive. In our cohort, the lowest effective daily dose for sustained benefit was 400 mg/day, but we noted that higher doses should be used in order to maximise the clinical response.

We conclude that patients with PDHC deficiency who present with relapsing ataxia or Leigh disease are more likely to respond to thiamine treatment than those who present with neonatal lactic acidosis and have structural abnormalities of the corpus callosum on imaging. However, responsiveness to thiamine cannot be reliably predicted based on these clinical and neuroimaging differences since some overlap between the two groups exists. We suggest that high-dose (>400 mg/day) thiamine treatment be initiated as soon as PDHC deficiency is suspected. Early treatment will quickly reverse the manifestations of thiamine deficiency and might prevent irreversible complications (e.g. contractures). Plasma thiamine concentration and mutation analysis of the *PDHAI* gene should be done as first-line investigations (along with the usual metabolites analysis), in order to avoid missing thiamine-responsive patients and misdiagnosing patients with secondary PDHC deficiency. If negative, enzymatic analysis, molecular analysis of other PDHC genes and a search for other diagnoses should be done. New molecular tests for defects in thiamine transport will enable the correct diagnosis in more patients who present with manifestations suggestive of PDHC deficiency.

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Compliance with Ethics Guidelines

Conflict of Interest

All the authors of this chapter declare that there are no conflicts of interest.

Details of the Contributions of Individual Authors

Sanne Van Dongen: Reviewed the literature, reviewed all patients' records, collected data into Excel spreadsheets, wrote first manuscript and corrected and rewrote subsequent versions. Reviewed the final version and approved it.

Ruth Brown: Performed mutation analysis, reviewed mutations results and participated in writing the methods, results and discussion sections. Critically reviewed the manuscript and approved its final version.

Garry Brown: Reviewed mutations results and participated in writing the methods, results and discussion sections. Critically reviewed the manuscript and approved its final version.

David Thorburn: Reviewed enzymology and mutation results and participated in writing the methods, results and discussion sections. Critically reviewed the manuscript and approved its final version.

Avihu Boneh: Initiated the study, supervised and reviewed all clinical and laboratory data collection and tabulation, participated in writing all versions of the manuscript and wrote the final version of the manuscript.

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