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Founder p.Arg 446* mutation in the *PDHX* gene explains over half of cases with congenital lactic acidosis in Roma children

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ABSTRACT

Investigation of 31 of Roma patients with congenital lactic acidosis (CLA) from Bulgaria identified homozygosity for the R446* mutation in the *PDHX* gene as the most common cause of the disorder in this ethnic group. It accounted for around 60% of patients in the study and over 25% of all CLA cases referred to the National Genetic Laboratory in Bulgaria. The detection of a homozygous patient from Hungary and carriers among population controls from Romania and Slovakia suggests a wide spread of the mutation in the European Roma population. The clinical phenotype of the twenty R446* homozygotes was relatively homogeneous, with lactic acidosis crisis in the first days or months of life as the most common initial presentation (15/20 patients) and delayed psychomotor development and/or seizures in infancy as the leading manifestations in a smaller group (5/20 patients). The subsequent clinical picture was dominated by impaired physical growth and a very consistent pattern of static cerebral palsy-like encephalopathy with spasticity and severe to profound mental retardation seen in over 80% of cases. Most patients had a positive family history.

We propose testing for the R446* mutation in *PDHX* as a rapid first screening in Roma infants with metabolic acidosis. It will facilitate and accelerate diagnosis in a large proportion of cases, allow early rehabilitation to alleviate the chronic clinical course, and prevent further affected births in high-risk families.

1. Introduction

Pyruvate dehydrogenase is the rate-limiting enzymatic step that links glycolysis to the tricarboxylic acid cycle and mitochondrial energy metabolism [1]. The pyruvate dehydrogenase complex (PDC) consists of three catalytic subunits (pyruvate dehydrogenase, E1; dihydrolipoamide transacetylase, E2; dihydrolipoamide dehydrogenase, E3), two regulatory subunits (E1 kinase and E1 phosphatase) and an E3-binding protein

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Table 1
Early clinical manifestations in *PDHX* p.Arg446* homozygotes.

Case no.	Family	Sex	Birth		Dysmorphic features	Age	Onset	Metabolic data at onset				Antenatal brain damage		
			At term	BW (g)			Manifestations	Blood lactate mmol/l	Urine lactate U/mol Cr	Alanine μmol/l	Urine 2-HO — butyrate U/mol Cr	Corpus callosum hypo-/aplasia	Periventricular cysts	Other lesions in the first 3 weeks after birth
1	A	M	+	3000	Epispadias, inguinal hernia	1 h	Lactic acidosis crisis	No data	27,780	3549 (P)	410	+	−	−
2	A	M	+	3000	−	8 d	Lactic acidosis crisis	No data	8002	2135 (P)	372	−	−	−
3	B[#]	M	+	3400	Ear malrotation, low umbilicus	16 d	Lactic acidosis crisis	11.1	63	386 (DBS)	32	−	+	Ventricular dilatation
4	C[#]	F	+	3150	−	16 d	Lactic acidosis crisis	11.0	11,793	No data	2200	+	+	Ventricular dilatation
5	D	F	+	2850	−	17 d	Lactic acidosis crisis	No data	3764	1322 (P)	1025	−	+	Ventricular septi
6	E	M	No data	2480	−	20 d	Lactic acidosis crisis	13.3	380	1099 (DBS)	75	−	−	−
7	E	M	+	2400	−	23 d	Lactic acidosis crisis	15.0	425	639 (DBS)	82	−	−	−
8	D	M	+	2800	−	29 d	Lactic acidosis crisis	No data	12,989	No data	3035	+	−	−
9	F	F	+	3000	−	25 d	Lactic acidosis crisis	No data	4614	No data	78	−	+	−
10	G	F	+	3200	−	2 m	Lactic acidosis crisis	7.3	No data	No data	No data	+	−	−
11	I[#]	F	+	3100	−	5 m	Lactic acidosis crisis	5.5	5584	No data	262	+	−	−
12	J	M	+	2800	Epicanthal folds	12 d	Lactic acidosis crisis	12.68	23,324	903 (P)	966	+	+	Ventricular dilatation
13	K	F	No data	No data	Facial dysmorphism, non-specified	2 m	Lactic acidosis crisis	No data	15,191	2942 (DBS)	838	+	−	−
14	L	M	+	2400	−	2 d	Lactic acidosis crisis	No data	3233	1072 (DBS)	482	−	−	Ventricular dilatation
15	M	F	+	3150	Hypertelorism, epicanthal folds	20 d	Vomiting, seizures, somnolence	No data at onset				+	−	−
16	M	M	+	3905	−	4 m	Developmental delay	No data at onset				−	−	No data
17	N	M	+	No data	Short philtrum	6 m	Developmental delay, seizures	No data at onset				−	−	No data
18	N	F	+	No data	Flat nasal bridge, hypertelorism	3 m	Developmental delay, seizures	No data at onset				−	−	No data
19	N	M	+	3050	Hypertelorism, megalocornea	6 m	Developmental delay, seizures	No data at onset				−	−	No data
20	O[#]	F	+	No data	−	6 m	Developmental delay	No data at onset				No data		

Legend: (+) — present; (−) — absent; [#] previous affected child deceased; BW — birth weight; h — hour, d — day, m — month, y — year; OH — hydroxy; DBS — dried blood spot; P — plasma; urine metabolites are represented in relative units (U): ratio of chromatographic peak area of metabolite to that of added internal standard (3-Phenylbutyric acid) per mol creatinine (Cr). Reference ranges of metabolites: blood lactate <2.2 mmol/l; DBS alanine — 114–577 (newborn), 92–290 (1 m–1 y) μmol/l; plasma alanine — 89–508 (newborn) μmol/l; urine lactate — 8–421 U/mol Cr; 2-hydroxybutyrate — 0–85 U/mol Cr.

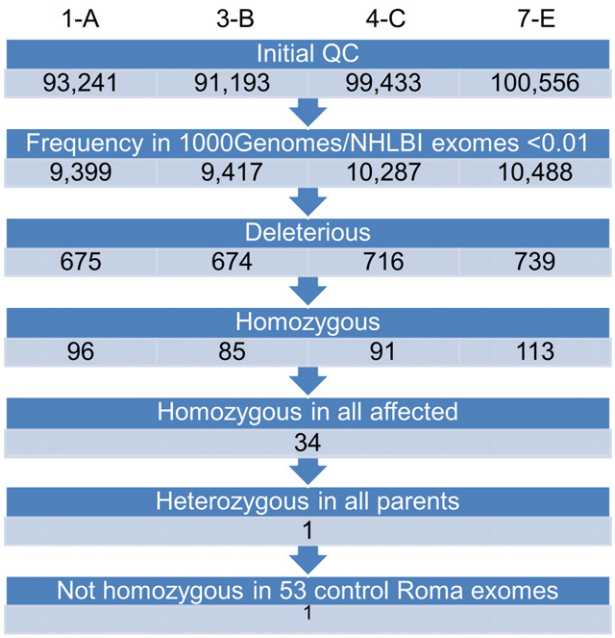

Fig. 1. Step-wise filtering of the variants identified by WES.

(E3BP) that binds E2 to E3. PDC deficiency is a major cause of congenital lactic acidosis (CLA) that leads to failure in energy production and developmental and encephaloclastic disorders of the central nervous system (reviewed in [1,2] and recently in [3–5]). The incidence and prevalence of PDC deficiency are not well defined and the large number of genes encoding PDC components implies mutation heterogeneity that requires extensive biochemical and genetic work-up leading to etiological diagnosis. Mutations in the *PDHX* gene, encoding E3BP, are the second most common cause of PDC deficit [3]. All are null-mutations, confined to individual families, that result in complete protein deficit and substantial reduction of PDC activity (reviewed in [6,7]).

Here we report a founder mutation in the *PDHX* gene, explaining over half of CLA cases in children of Roma ethnicity. We present data on the phenotypic features and evolution of the disorder in 20 patients homozygous for the mutation, the largest group of genetically homogeneous affected subjects reported to date. Our findings provide a simple test for fast-track diagnosis in this ethnic group that can substitute for the lengthy process of clarifying the variable causes of metabolic crisis in the newborn.

2. Subjects and methods

2.1. Subjects

The study included 56 participants from 23 nuclear Roma families from Bulgaria (3 of these belonged to an extended kindred): 31 individuals with diagnosed or suspected CLA and 25 healthy relatives. Seventeen affected infants were referred by the Department of Pediatrics and Medical Genetics at Plovdiv Medical University, and eight by the National Genetic Laboratory. Six additional cases (four cousins and two siblings from a different family), diagnosed as hereditary spastic paraplegia with mental retardation, were referred by the Neurology Department of the Medical University-Sofia because of hyperlactataciduria detected during previous hospital admissions in three of these patients. In the course of the study, 21 CLA patients of unspecified ethnicity from Hungary were tested for the presence of the *PDHX* mutation.

Written informed consent has been provided by all participating subjects or their guardians. The study complies with the ethical guidelines of the institutions involved.

Carrier frequency was estimated in a panel of 657 de-identified ethnically matched controls and 53 exome controls, representing Roma groups from the Balkan, Vlax and Central/West European migration categories [8].

2.2. Clinical data

Clinical, metabolic, neurophysiological and imaging (brain ultrasound, CT and MRI) data were collected from hospital records and during follow-up visits.

2.3. Biochemical analyses

Routine blood and urine analyses assessed blood, liver, and kidney function. Urine organic acids were analyzed by gas chromatography–mass spectrometry (Agilent Technologies 7890A GC/5975C MSD) after organic solvent sample extraction with trimethylsilylation. Amino acid analysis in dried blood spots was performed by electrospray ionization–tandem mass spectrometry after organic solvent sample extraction without derivatization, on a Waters TQ Detector equipped with Waters 1526 μBinary HPLC Pump, using manufacturer kits (Chromsystems). Plasma amino acids were analyzed by reversed phase high performance liquid chromatography with fluorescence detection (Waters HPLC system). PDH complex enzyme activity was measured in cultured skin fibroblasts (patient 3) using 2 mg of cell lysates with the PDH Enzyme Activity Microplate Assay Kit (Abcam) following the manufacturer's instructions.

2.4. Genetic analyses

DNA was extracted from blood in most cases except 10 affected infants, where archived urine (7 cases) or Guthrie cards (3 cases) were the only available biological material.

Whole exome sequencing (WES) was performed at the University of Queensland Centre for Clinical Genomics on DNA samples from three trios and a singleton (affected child and parents from families A, B and C and patient 7 in Table 1) from the Plovdiv sample. We used the SeqCap Roche Human Exome V3 capture system (Roche NimbleGen, Madison, WI, USA) and the HiSeq2000 platform (Illumina, San Diego, CA, USA). Initial data processing was done as described previously [9,10]. We used 6491 markers, extracted from WES data at HapMap Phase II SNP positions [10,11] to estimate inbreeding coefficients [12] and relatedness [13]. The search for the disease-causing mutation focused on variants with a quality score ≥20 and coverage ≥4×, located outside of segmental duplications and simple repeats. The step-wise filtering

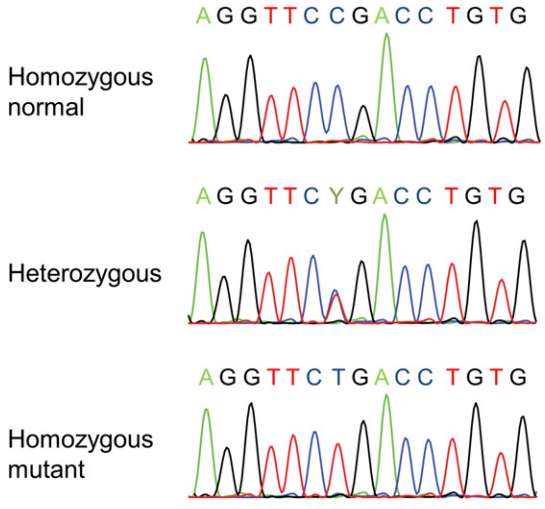

Fig. 2. Sanger sequencing of *PDHX* with the three c.1336C>T genotypes.

criteria included: a) allele frequency ≤1% in the 1000Genomes (http://www.1000genomes.org/) or NHLBI (http://evs.gs.washington.edu/EVS/) Exome Sequencing projects; b) "deleteriousness" predictions (Polyphen2 [14] scores >0.8 and SIFT [15] scores ≤0.05), splice-site (±15 nt), nonsense, non-stop, and small in-frame or frame-shift in/dels; c) shared homozygosity between affected individuals; d) heterozygosity of the parents; e) no homozygous subjects in the in-house Roma control exomes.

Mutation screening in the panel of population controls was performed with a custom-designed TaqMan® assay with forward and reverse primers CCTCAGGCCTGCATTTTGG and CATTTCCCTCTTCATCCTCAGTGA, respectively, and reporters CTTCAGCACAGGTCGGA and CTTCAGCACA GGTCAGA. The heterozygotes identified by the screening assay were confirmed by Sanger sequencing.

Bidirectional Sanger sequencing of PCR-amplified fragments was performed using BigDye terminator v3.1 (Applied Biosystems, Mulgrave, VIC, Australia) with primers PDHXfwd AAGGACATGCCTCC TTCAGA and PDHXrev TTCGTCATCAACCACTCGAC.

DNA from urine was obtained from 50 ml urine centrifuged at 1000 *g* and the sediment stored and transported in Thermo Scientific™ AssayAssure™ Universal Urine Collection Tubes. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, VIC, Australia).

3. Results

3.1. Genetic findings

3.1.1. WES data analysis reveals inbreeding and identifies a truncating mutation in PDHX

The F coefficients of the four affected individuals with exome data were estimated at 0.040–0.079, corresponding to the offspring of 1st cousins to 1st cousins once removed. Relatedness analysis revealed distant relationships, 2nd cousins or lower between parents within and between families (mean proportion of alleles identical by descent 0.029 ± 0.0106).

Based on the common origins and evidence of inbreeding, our search for the disease-causing mutation was based on the assumption of shared homozygosity for a rare pathogenic variant. Exome-wide, a single variant passed all filtering criteria (Fig. 1): NM_003477.2: c.1336C>T in the *PDHX* gene, predicted to result in a premature termination codon in the last exon, p.Arg446*.

Sanger sequencing (Fig. 2) confirmed the WES findings: the four patients were homozygous for the mutation and the six parents were carriers.

3.1.2. p.Arg446* is a founder mutation that explains 60% of cases of congenital lactic acidosis in Roma children

Mutation screening of 710 population controls identified five carriers, three from Balkan Roma groups in Bulgaria, and one each from Romania and Slovakia, translating to an overall carrier rate of about 1 in 150 individuals in the general Roma population. No heterozygotes were detected among the Vlax Roma in Bulgaria.

Next, we used Sanger sequencing to examine the presence of the p.Arg446* mutation in the entire group of affected individuals and their relatives. Homozygosity for the mutation was revealed in 19 out of the 31 Roma patients from Bulgaria, thus explaining around 60% of congenital lactic acidosis in this ethnic group. One homozygote was also detected in the Hungarian sample, but further conclusions on its frequency cannot be drawn due to the lack of data on ethnicity.

The 20 p.Arg446* homozygous patients belonged to 15 nuclear families, two of which were related (family N in Table 1). Five families were represented by sib pairs; patient 19 and siblings 17 and 18 from family N were 1st cousins once removed. Of the remaining nine cases, four had a deceased affected sibling. Thus 9/14 families had more than one affected child (Table 1).

The residual group of mutation-negative patients reveals genetic heterogeneity. One affected child with typical manifestations of neonatal onset lactic acidosis crisis was found to be a p.Arg446* carrier, a coincidental finding that cannot explain the disease. Heterogeneity can exist even within the same extended family: in family N, 3/4 affected cousins were p.Arg446* homozygots while the fourth was not even a carrier. The mutations in this group of patients await clarification.

3.2. Clinical features

3.2.1. Lactic acidosis crisis in infancy was the most common initial presentation

The findings are summarized in Table 1. The predominant clinical phenotype was that of a lactic acidosis crisis occurring in the newborn period (11 cases) or early infancy (3 cases). Both sexes were equally represented. Patient no. 15 is likely to belong to this group as well, based on onset with vomiting, seizures and somnolence, however no further analyses were performed at the time. In this patient, hyperlactataciduria (1529 U/mol Cr) and hyperalaninemia (852 μmol/l) were first determined at age 1 y 9 m.

The presenting symptoms in this group were variable combinations of vomiting, failure to thrive, apnea/grunting, hypotonia, seizures, stupor and coma. In 5 cases the metabolic crisis had been provoked by infection. Upon admission, all patients had decompensated metabolic acidosis (pH 6.9 to 7.36, BE values −4.7 to −22). Blood lactate levels varied between 5.5 and 15 mmol/l, urine excretion of lactate and 2-hydroxybutyric acid ranged from borderline to massive, with variable increased excretion of Krebs cycle metabolites, and ketoacids found occasionally. Blood amino acid analysis showed the invariable presence of hyperalaninemia. Non-specific pathological findings commonly included proteinuria and hematuria. PDC activity in fibroblasts was 20% of control values. Data on pyruvate concentrations were not available.

In a smaller group of patients (nos. 16 to 20), the reason for seeking medical attention included delayed psychomotor development and/or seizures at age 3–6 months. In this group, hyperlactatemia had been diagnosed at a later age (patients 17,18, 20).

While all children had been born at term, birth weight (2980.31 ± 379.76 g) was below the population mean (4000 ± 600 g male and 3700 ± 600 female) [16]. Microcephaly (head circumference < X − 2 SD) in the neonatal period was observed in 6 (nos. 2, 3, 6, 7, 9, and 15) out of 7 children with available head circumference data from that period.

Variable minor malformations or dysmorphic features of no consistent pattern were described in 8 patients (5 male).

Antenatal brain damage was commonly observed — found in 12 of the 15 patients where data were available. Aplasia or hypoplasia of corpus callosum was the most consistent abnormality, found in 8 children (5 females). Other common findings included periventricular cysts and atrophic ventricular dilatation.

3.2.2. Intellectual deficit and spasticity dominate the evolution of the disease

The phenotypic features beyond the first months of life are summarized in Table 2.

Most affected children survived infancy. Death occurred during the first year of life in two patients, another died at age 11 y, and a fourth patient died at an unspecified age. The oldest living patients in the study were 22 and 25 y old.

Physical development was impaired in most cases, affecting height, weight and head circumference. Microcephaly was present in 10 of the 14 children with known last visit measurements. Mean head circumference was X − 3.50 SD.

The frequency of lactic acidosis crises decreased gradually, with none registered after age 5 y. Moderate hyperlactacidemia persisted with values ranging between 2.3 and 5.5 mmol/l in nine children for

Table 2
Phenotype evolution in patients homozygous for *PDHX* p.Arg446*.

Case #	Age at most recent clinical assessment	Physical development (SD)			Intellectual development DQ/IQ	Neurological findings					Postnatal chronic brain lesions	Blood lactate (mmol/l)/at age
		Height	Weight	Head circumference		Motor	Dystonia/chorea	Epileptic seizures	Visual pursuit	Hearing (audiopalpebral reflex)		
1	9 y	−1.31	−0.83	−2.01	21	Spastic diplegia	Focal paroxysmal, since 3 y	Neonatal seizures; EEG: biF & generalized SW at 5 y, normal at 6 & 8 y	+	+	Bilateral hyperintense lesions in globus pallidus, cortical and periventricular brain atrophy, frontal and occipital periventricular leukoencephalopathy at 5 and 8 y on MRI	3.5/5 y 3 m
2	Died at 32 d											
3	4 y	−0.22	−1.82	−2.67	25	Spastic quadriparesis	−	GTCS at 12 m; EEG: abnormal background activity at 1 & 2 y	+	+	Cortical and periventricular brain atrophy after 4 m on US	4.7/2 y 8 m
4	5 y	−3.85	−4.45	−12.2	6	Spastic quadriparesis	−	SGTCS since 11 m; EEG: R F slow SW at 5 m, multifocal SW at 2 y	−	−	Hyperechogenicity of basal ganglia and cortical and periventricular brain atrophy after 3 m on US	No data
5	Died, age unspecified	No data	No data	No data	"Low"	Spastic quadriparesis	−	GTS; EEG: burst-suppression pattern	No data	No data	Multicystic encephalomalacia after 22 d on US	2.94/8 m
6	2 y	−3.99	−3.81	−4.41	29	Hypotonia	−	No seizures; EEG: abnormal background activity at 8 m	−	+	Cortical and periventricular brain atrophy after 1 m on US	No data
7	4 y	−2.96	−2.56	−3.46	25	Hypotonia	−	SGCS & infantile spasms at 2 y; EEG: normal at 8 m, multifocal SW 2 y, R C–T SW at 5 y	−	+	Cortical and periventricular brain atrophy after 4 m on US and MRI; normal MR spectroscopy at 1 y	2.74/2 y 4 m
8	9 y	−3.43	−1.89	−0.44	<20	Spastic diplegia	Frequent short dystonic postures	No seizures; EEG: low voltage at 1 m, C–T SW at 9 y	+	+	Cortical and periventricular brain atrophy after 1 m on US; periventricular leukoencephalopathy at 9 y on MRI	2.3/9 y
9	14 y		No data		<11	Spastic quadriparesis	−	Neonatal seizures, no data after 5 m; EEG: biF & R C sharp waves at 1 m; hypsarrhythmia at 5 m	No data	No data	Cortical and periventricular brain atrophy after 5 m on US	No data
10	7 y	−2.39	−1.09	−3.01	<30	Spastic diplegia	Hemi-dystonic (R > L), since 5 y	SGTCS at 5 y; EEG: normal at 5 & 6 y; rhythmic theta at 7 y	+	+	Hyperechogenicity of basal ganglia; cortical and periventricular brain atrophy after 2 m on US	2.6/7 y

11	10 y	−5.6	−3.13	−7.54	5	Spastic quadriparesis	−	SGTCS since 5 m; EEG: L C–P–T SW at 5 y; R C–P–O spikes and SW at 10 y	+	+	Cortical and periventricular brain atrophy at 10 y on MRI	5.5/10 y
12	Died at 40 d											
13	No data											
14	1 y 1 m	No data	−1.4	−1.5	30	Spastic quadriparesis	−	PMS and GTCS since 6 m; EEG normal at 1 y	+	+	Periventricular brain atrophy at 10 m on CT scan	No data
15	1 y 6 m died 11 y	−1.8	−0.5	−1.9	<30	Spastic quadriparesis	−	Infantile spasms; EEG: R P sharp waves	+	+	Cortical atrophy at 4 m on CT; Cortical and periventricular brain atrophy and subcortical leukoencephalopathy at 7 y on MRI	No data
16	1 y	No data	+0.25	−0.5	<30	Spastic quadriparesis	−	−	+	+	Cortical and periventricular brain atrophy at 1 y on CT	No data
17	14 y 25 y	−2.44–2.54	−2.9	−2.7–2.8	<30	Spastic quadriparesis	−	GTCS since 6 m; EEG: abnormal slow background activity at 14 y	+	+	Cortical and periventricular brain atrophy and subcortical leukoencephalopathy at 14 y on MRI	3.14/14 y
18	11 y 22 y	−2.75–2.80	−2.2	−4.4–4.5	<20	Spastic quadriparesis	−	GTCS between 3 m and 5 y; EEG: abnormal slow background activity at 11 y	+	+	Cortical and periventricular brain atrophy, periventricular leukoencephalopathy at 11 y on MRI	4.06/11 y
19	4 y	+1.0	No data	−2.2	<30	Axial hypotonia	Choreiform movements in the UL	CPS and SGTCS since 2 y	−	+	Cortical brain atrophy at 3 y on CT	No data
20	4 y	No data	No data	No data	<30	Spastic quadriparesis	−	−	+	No data	No data	No data

Legend: (+) — present; (−) — absent; R — right, L — left, UL — upper limbs, d — day, m — month, y — year, F — frontal, C — central, P — parietal, C–T — centrotemporal, C–P–T — centroparietotemporal, C–P–O — centroparietooccipital, CPS — complex partial seizures, GTCS — generalized tonic clonic seizures, SGTCS — secondary generalized tonic clonic seizures; US — brain ultrasound.

Table 3
Summary of clinical findings in patients homozygous for *PDHX* p.Arg446*.

Patient	Intrauterine growth retardation	Dysmorphic features	Antenatal brain lesion	Lactic acidosis crisis in newborn or in early infancy	Recurrent lactic acidosis crisis	Impaired physical development	Intellectual deficit	Spasticity/ Cerebral palsy-like	Paroxysmal dystonia	Epileptic seizures	Sensory impairment	Relapsing ataxia	Acute/recurrent brainstem dysfunction
1	−	+	+	+	−	±	+	+	+	+	−	−	−
3	−	+	+	+	+	+	+	+	−	+	−	−	−
4	−	−	+	+	+	+	+	+	−	+	+	−	−
5	−	−	+	+	No data	+	+	+	No data	+	+	−	−
6	+	−	−	+	+	+	+	−	−	−	+	−	−
7	+	−	−	+	+	+	+	−	−	+	+	−	−
8	−	−	+	+	−	+	+	+	+	−	−	−	−
9	−	−	+	+	−	No data	+	+	−	+	−	−	−
10	−	−	+	+	+	+	+	+	+	+	−	−	−
11	−	−	+	+	+	+	+	+	−	+	−	−	−
14	+	−	+	+	−	−	+	+	−	+	−	−	−
15	−	+	+	±	−	−	+	+	−	+	−	−	−
16	−	−	No data	−	−	−	+	+	−	−	−	−	−
17	No data	+	No data	−	−	+	+	+	−	+	−	−	−
18	No data	+	No data	−	−	+	+	+	−	+	−	−	−
19	−	+	No data	−	−	−	+	−	−	+	+	−	−
20	No data	−	No data	−	−	No data	+	+	No data	No data	No data	No data	No data
2	−	−	−	+	Died in infancy								
12	−	+	+	+	Died in infancy								
13	No data	+	+	+	No data								

Legend: (+) – present; (−) – absent, (±) – probably present.

whom data were available at age 8 months to 14 y. The glucose loading test [17] performed in 7 children (nos. 1, 3, 4, 6, 7, 8, 11) showed a reliable blood lactate increase in all.

The clinical phenotype was dominated by central nervous system signs. Intellectual deficit was invariably present, ranging from severe to profound. Spasticity was a typical finding, observed in 14 of the 17 cases where information was available. Dystonic attacks were rare, and present in only three patients with cerebral palsy (CP)-like motor abnormalities.

Four patients were virtually blind (nos. 4, 6, 7 and 19). Visual evoked potentials were tested in three patients and were abnormal in two (nos. 5 and 6) and normal in patient nos. 7 at age 7 m. No abnormalities in eye fundi were detected in any of the 16 patients examined. Abnormal hearing, clinically or on brainstem auditory evoked response, was found in 4 patients (nos. 2, 4, 5 and 6).

Epileptic seizures were observed in 13 patients; two (nos. 6 and 8) had abnormal EEG recordings without history of seizures.

EMG, performed in four patients, showed axonal neuropathy at 8 y in patient no. 1 after being normal at 5 y, myopathic changes in patient no. 11 at 10 y, and normal findings in patient nos. 7 at 2 y and no. 15 at 7 y. No EMG data are available for patient 19, who presented with generalized hyporeflexia.

Cortical brain atrophy and ventricular enlargement were the dominant postnatal brain lesions, observed in 15 patients. Basal ganglia involvement was found in three patients.

4. Discussion

PDHX defects are the second most common cause of CLA after *PDHA1* mutations, yet the overall number of published cases is less than 30, with diverse mutations and ethnic origins (most recently reviewed in [7]). Here, we present a genetically homogeneous group of 20 Roma patients homozygous for a single ancestral mutation in the *PDHX* gene, p.Arg446*. The age range of the patients and the availability of clinical information at various time points allowed us to summarize the pattern of presentation and evolution of the disease (Table 3).

The phenotype was relatively homogeneous with no obvious differences between the sexes. Antenatal brain damage and microcephaly were common findings. The presentation was usually emergency hospitalization due to lactic acidosis crisis in the first days or months of life. Most patients survived into childhood. The subsequent clinical picture was that of impaired physical growth and a consistent pattern of static cerebral palsy-like encephalopathy with spasticity and severe to profound mental retardation. Epileptic seizures occurred in over half of cases. Sensory impairment (vision and auditory function) was relatively common. While no acute/recurrent episodes of brainstem dysfunction were documented, six patients (Table 2) developed signs and symptoms consistent with Leigh syndrome: neuroimaging evidence of basal ganglia involvement in three subjects, muscle hypotonia in three, and movement disorders in four (dystonia in three and choreiform movements in one). Mild axonal neuropathy was found in one patient. Relapsing ataxia was altogether absent.

The clinical features of our p.Arg446* homozygotes agree with previously published findings in patients with *PDHX* mutations (reviewed in [5–7,18]) as regards onset in the first months of life with lactic acidosis, survival beyond the 1st year and psychomotor retardation. The uniformity and characteristics of the neurological phenotype in our patients contrast with other studies. CP-like spasticity was present in 14/17 of our cases, but was relatively rare in other descriptions: 0/19 patients [6], 2/11 [18] and 4/24 [7], with the exception of [5] where 5/5 patients displayed a CP-like clinical picture with dystonia. Epileptic seizures were also more common, occurring in 13/16 of our patients, while in only 4/19 patients [6], 2/11 [18], 2/5 [5] or 4/24 cases [7].

The p.Arg446* mutation has been reported previously in three patients of unspecified ethnicity in the UK and France [6,7]. It would be of interest to know if these are Roma or Indian/Pakistani families

sharing the same ancestral mutation, as shown in other disorders [19]. Our study shows that *PDHX* p.Arg446* is the major cause of primary lactic acidosis in Roma children. It accounted for around 60% of affected subjects in the study and for 27% of a total of 69 cases of lactic acidosis (regardless of ethnicity) referred to the National Genetic Laboratory during the period 1996–2013. While the overall carrier rate is not high compared to other recessive founder mutations in the Roma [19], the detection of an affected homozygote in Hungary and of carriers in Romania and Slovakia indicates that the mutation is wide spread and that affected births can occur anywhere in Europe. The finding of relatedness between affected families that are unaware of each other's existence points to local founder effect and on-going inbreeding leading to increased risk in some Roma communities. Inter-family relatedness, which we have not observed in other studies [13], may also contribute to the homogeneity and characteristic features of the phenotype — in addition to being homozygous for the same ancestral mutation, the patients are more likely to share a similar genetic background.

Establishing the cause of metabolic acidosis in a critically ill newborn involves a series of metabolic analyses, followed in the case of lactic acidosis by measurements of PDC activity, protein levels of its subunits and sequencing of the gene(s) presumed to be involved. Given the high proportion of *PDHX* p.Arg446* homozygotes among Roma babies with lactic acidosis, we propose mutation testing as a first-tier shortcut that can greatly simplify and facilitate the diagnosis and allow early dietary and rehabilitation measures to alleviate the severely disabling subsequent chronic course of the disease. Although the efficacy of dietary treatment in PDHc deficit has not been documented in prospective controlled trials [3], a beneficial effect of ketogenic diet on childhood epilepsy and paroxysmal dystonia has been reported in patients with PDHX mutations [5,6,20]. In addition to the correction of acidosis during the early LA crises in infancy, the introduction of a ketogenic diet may modify the clinical course of the disease, at least in patients that have the above neurological manifestations. Early initiation of rehabilitation measures is at least as important, given the high frequency of spasticity in the CP-like phenotype.

Further, the early diagnostic “clue” of lactic acidosis attacks is gradually lost in early childhood (this study, [7,21]) and replaced by non-specific phenotypic features that make the metabolic and genetic diagnosis more problematic and easier to miss. Including the p.Arg446* mutation in the diagnostic workup of the Roma patient with growth retardation, “cerebral palsy” and mental retardation may not give a high yield of positive results, but will speed up the procedure in some cases and, with time, will provide information on the contribution of CLA to the poorly defined category of “cerebral palsy” in this ethnic group. Furthermore, over 60% of families participating in this study had more than one affected child. Early selective screening will allow genetic counseling and help the prevention of further affected births.

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Conflict of interest

The authors declare no conflict of interests.

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