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Vieira, Päivi

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ORIGINAL ARTICLE

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Cytosolic phosphoenolpyruvate carboxykinase deficiency: Expanding the clinical phenotype and novel laboratory findings

Päivi Vieira[1,2] | **Irina I. Nagy**[3] | **Elisa Rahikkala**[2,4,5] | **Marja-Leena Väisänen**[3] | **Katariina Latva**[6] | **Kari Kaunisto**[1,2] | **Pekka Valmari**[7] | **Riikka Keski-Filppula**[2,4] | **Maria K. Haanpää**[5,8] | **Virpi Sidoroff**[9] | **Päivi J. Miettinen**[10] | **Tuula Arkkola**[1] | **Marja Ojaniemi**[1,2] | **Matti Nuutinen**[1,2] | **Johanna Uusimaa**[1,2] | **Päivi Myllynen**[3]

[1]Clinic for Children and Adolescents, Oulu University Hospital, Oulu, Finland

[2]PEDEGO Research Unit and Medical Research Center, Oulu University Hospital and University of Oulu, Oulu, Finland

[3]Department of Clinical Chemistry, Cancer and Translational Medicine Research Unit, Medical Research Center, University of Oulu and Northern Finland Laboratory Centre NordLab, Oulu University Hospital, Oulu, Finland

[4]Department of Clinical Genetics, Oulu University Hospital, Oulu, Finland

[5]Institute of Biomedicine, University of Turku, Turku, Finland

[6]Department of Pediatrics, Päijät-Häme Central Hospital, Lahti, Finland

[7]Department of Pediatrics, Lapland Central Hospital, Rovaniemi, Finland

[8]Department of Clinical Genetics, Turku University Hospital and University of Turku, Turku, Finland

[9]Department of Pediatrics, North Karelia Central Hospital, Joensuu, Finland

[10]New Children's Hospital, Helsinki University Hospital, Pediatric Research Center, Helsinki, Finland

Correspondence
Päivi Vieira, Clinic for Children and Adolescents, Oulu University Hospital, PO BOX 23, Oulu 90029, Finland.
Email: paivi.vieira@fimnet.fi

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Abstract

Cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C) deficiency due to the homozygous *PCK1* variant has recently been associated with childhood-onset hypoglycemia with a recognizable pattern of abnormal urine organic acids. In this study, 21 children and 3 adult patients with genetically confirmed PEPCK-C deficiency were diagnosed during the years 2016 to 2019 and the available biochemical and clinical data were collected. All patients were ethnic Finns. Most patients (22 out of 24) had a previously published homozygous *PCK1* variant c.925G>A. Two patients had a novel compound heterozygous *PCK1* variant c.925G>A and c.716C>T. The laboratory results showed abnormal urine organic acid profile with increased tricarboxylic acid cycle intermediates and inadequate ketone body production during hypoglycemia. The hypoglycemic episodes manifested predominantly in the morning. Infections, fasting or poor food intake, heavy exercise, alcohol consumption, and breastfeeding were identified as triggering factors. Five patients presented with

Päivi Vieira and Irina I. Nagy contributed equally as first authors to this study.

Johanna Uusimaa and Päivi Myllynen contributed equally as senior authors.

neonatal hypoglycemia. Hypoglycemic seizures occurred in half of the patients (12 out of 24). The first hypoglycemic episode often occurred at the age of 1-2 years, but it sometimes presented at a later age, and could re-occur during school age or adulthood. This study adds to the laboratory data on PEPCK-C deficiency, confirming the recognizable urine organic acid pattern and identifying deficient ketogenesis as a novel laboratory finding. The phenotype is expanded suggesting that the risk of hypoglycemia may continue into adulthood if predisposing factors are present.

KEYWORDS

citric acid cycle, gluconeogenesis, hypoglycemia, pediatrics, *pck1*, seizure

1 | INTRODUCTION

Phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) is the rate-controlling enzyme in the metabolic pathway of gluconeogenesis. PEPCK has two isoforms, cytosolic (PEPCK-C) and mitochondrial (PEPCK-M). Cytosolic phosphoenolpyruvate carboxykinase deficiency (MIM 261680) due to *PCK1* (MIM 614168) homozygote variant was recently demonstrated to be responsible for childhood-onset symptomatic hypoglycemia.[1] The patients had transiently elevated liver transaminase levels and abnormal amounts of tricarboxylic acid (TCA) cycle metabolites, especially fumarate, in the urine. The symptoms were often triggered by infections. The metabolic abnormalities normalized within 1 to 2 months of the hypoglycemic episode.

Gluconeogenesis is required for the production of endogenous glucose at times of inadequate carbohydrate intake, with pyruvate, lactate, and glucogenic amino acids used as major hepatic substrates. PEPCK-C catalyzes the conversion of oxaloacetate (OAA) to phosphoenolpyruvate in the initial step of gluconeogenesis in the cytosol; OAA is formed from pyruvate and TCA cycle intermediates. The liver is the main organ for gluconeogenesis, and enzyme activity of PEPCK-C is high in the liver.[2,3] Besides the role in gluconeogenesis, PEPCK is important in cataplerosis and other metabolic processes involving hepatic and intestinal energy metabolism, as shown in vitro and in mouse models.[4-7] In the absence of hepatic PEPCK-C, activity of the TCA cycle is impaired, causing deficient fatty acid oxidation with secondary accumulation of triglycerides in liver cells.[8-10]

Cytosolic PEPCK deficiency is a rare inborn error of metabolism, with less than 10 genetically confirmed cases described in the literature.[1,11-15] Additionally, there are some earlier reports based on biochemical data only.[16-19] Here, we expand the clinical phenotype and laboratory findings of cytosolic PEPCK deficiency with the detailed data of 21 novel and three previously published patients. Furthermore, this study is the first to report adult patients with this disorder.

2 | METHODS

2.1 | Patients

Clinical and biochemical data was collected from 21 pediatric patients, and clinical data from three adult patients with symptomatic hypoglycemia diagnosed with genetically confirmed PEPCK-C deficiency in five Finnish hospitals (Oulu University Hospital, Lapland Central Hospital, Päijät-Häme Central Hospital, New Children's Hospital of Helsinki University Hospital, and North Karelia Central Hospital) during 2016 to 2019. All patients were ethnic Finns.

2.2 | Biochemical and genetic analyses

All existing laboratory data of the PEPCK-C deficient children were collected, but not all parameters were available from every patient. Basic biochemical analyses from blood and urine (see details in Supporting Information) were available from 19/24 PEPCK-C deficient patients, out of which 11/19 were under 3 years of age, and 8/19 between 3 and 8 years at the time of the diagnostic workup. Amino acid and organic acid analyses were available from 15/21 PEPCK-C deficient pediatric patients. Results from fasting tolerance tests were available for 11/21 pediatric patients. The fasting was terminated according to the age (1-2 years 18 hours; 2-8 years 20 hours; older than 8 years 24 hours) or when symptomatic hypoglycemia with blood glucose <3 mmol/L (<54 mg/dL) was reached.[20] The acute hypoglycemia and fasting tolerance test results were compared to reference values for symptomatic hypoglycemic pediatric patients

in the NordLab laboratory at Oulu University Hospital and from the literature.[21,22] Laboratory results were reviewed for the timing of the sample relative to the confirmed hypoglycemia. For detailed information on biochemical, genetic and statistical analyses see Supporting Information.

3 | RESULTS

3.1 | Clinical data

The clinical data of 21 novel cases and three previously published patients[1] are presented in Table 1. Five patients had hypoglycemia during the neonatal period (B-Glucose <2.6 mmol/L or 46.8 mg/dL) Half of the patients (12/24) presented around 1 year of age and most patients had several hypoglycemic episodes. Hypoglycemic seizures were reported in 12/24 patients; one of the patients had status epilepticus with a blood glucose value of 1.6 mmol/L (28.8 mg/dL). Electroencephalography (EEG) was performed on three patients after a seizure, and two of those were abnormal with focal slowing. Six patients received brain magnetic resonance imaging and one patient received brain computed tomography, all with normal results. All but one patient (F4P2) had normal cognition. Height and weight of all patients were within normal limits.

Many hypoglycemic episodes had an obvious triggering factor like gastroenteritis or febrile illness. In some cases, severe hypoglycemia with B-Glucose values of 1.2-1.8 mmol/L (21.6-32.4 mg/dL) occurred in the morning after a normal dinner and evening snack. However, many parents did mention a physically active day or poorer than normal eating during the previous evening. Hypoglycemic episodes became less frequent with age, but did occur beyond childhood. Adult patients reported alcohol consumption, often related to small doses of alcohol, and rigorous exercise as triggering factors. One female adult patient (F1P2) had symptoms from early childhood but her hypoglycemic episodes were most frequent during pregnancy and lactation, and occurred multiple times in the morning after breastfeeding at night. She was barely able to treat the symptoms at home without hospitalization.

After the first hypoglycemic episode leading to hospitalization, the patients received a glucometer/glucose sensor and were advised to avoid fasting and maintain sufficient carbohydrate intake. This included eating regular meals every 3 to 4 hours, with a special emphasis on the evening meal before bed. Five of the pediatric patients used cornstarch in the evenings, while the rest were recommended glucose polymer powder, as different regimens were used in different hospitals. Information on the glucose polymer emergency regimen (ER) was given by the dietitian to provide energy during illness or any physiological stress situation with reduced dietary carbohydrate intake. The ER was based on the standard emergency regimen guidelines,[23,24] see Table S1. No dietary restrictions were in place. After the diagnosis of PEPCK-C deficiency was established, the instructions of extra carbohydrate intake in the evenings, during illness or vigorous exercise were reinforced. The patients were also informed about the risks involved with alcohol consumption. Recorded hypoglycemic episodes were fewer after the genetic diagnosis was established and the abovementioned measures were discussed and implemented. Occasionally, a trigger such as gastroenteritis led to hypoglycemia in children despite precautions. Ten such episodes with blood glucose ranging from 1.7 to 2.8 mmol/L (30.6-50.4 mg/dL) were recorded in 4/21 pediatric patients.

3.2 | Biochemical data

During acute hypoglycemic episodes and fasting test, PEPCK-C deficient children presented with low glucose values, moderately high free fatty acid (FFA) mobilization but relatively low 3-hydroxybutyrate (3-OHBA) production (Table 2, Figure 1A,B). Hypoketotic response to hypoglycemia was observed in 13/15 PEPCK-C deficient children from which biochemical data were available. Only one patient (F15P1) presented with normal ketotic response. Blood gas analysis revealed moderately high lactate with respiratory compensated metabolic acidosis (Table 2, Figure 1C). Plasma alanine aminotransferase (ALT) was mildly elevated in most of the children during acute hypoglycemia (Table 2) and normalized within 1 month follow up (25 ± 14 U/L, $n = 10$). Two children had high hepatic transaminases for months (F3P1 ALT 43-316 U/L; F6P1 ALT 142-1429 U/L). Patient F3P1 also had abnormal coagulation test results (activated partial thromboplastin time APTT 44-47 seconds, normal 25-31 seconds) and fatty liver finding on ultrasound, which persisted for several months.

Blood glucose values were significantly lower during acute hypoglycemic episodes than during fasting tolerance test in PEPCK-C deficient patients, while ALT enzyme values were significantly higher after acute hypoglycemia. Lactate, 3-OHBA, FFA, and blood gas results were similar in children with PEPCK-C deficiency during acute hypoglycemia and fasting test (Table 2, Figure 1). Hormonal responses (insulin, ACTH, cortisol), total and free serum carnitine levels were normal.

TABLE 1 Clinical and genetic phenotype of patients with *PCK1* variants

Family (F), patient (P)	Gender	Age, years	Neonatal hypoglycemia	Age at first hypoglycemia after neonatal period, years	Age at latest hypoglycemia, years	Triggering factors	Seizure/ hypoglycemic coma	Gene variant zygosity	Nucleotide change	Amino acid change
F1P1	M	32	No	13	25	Infections in childhood, alcohol	No	Hom	c.925G>A	p.Gly309Arg
F1P2	F	31	Yes	4	31	Fasting, infections, alcohol, physical exercise, pregnancy, breastfeeding	Coma	Hom	c.925G>A	p.Gly309Arg
F1P3	F	8	Yes	1.2	6	Gastroenteritis	No	C/H	c.925G>A c.716 C>T	p.Gly309Arg p.Ser239Leu
F1P4	M	2	Yes	Asymptomatic[b]	0.1	Not applicable	No	C/H	c.925G>A c.716 C>T	p.Gly309Arg p.Ser239Leu
F2P1	M	35	No	Childhood	15	Poor food intake, alcohol	No	Hom	c.925G>A	p.Gly309Arg
F2P2	M	2.8	No	0.9	2	Infections, poor food intake	No	Hom	c.925G>A	p.Gly309Arg
F3P1[a]	F	8	Yes	1	7	Infections, poor food intake the preceding evening	No	Hom	c.925G>A	p.Gly309Arg
F3P2[a]	M	6	No	1	5	Poor food intake the preceding evening	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
F4P1	F	15	No	8	14	Poor food intake	Coma and sz	Hom	c.925G>A	p.Gly309Arg
F4P2	F	11	No	3.7	7	Infections, poor food intake	Coma and sz	Hom	c.925G>A	p.Gly309Arg
F5P1	F	10	No	1.5	3	Gastroenteritis, febrile illness	No	Hom	c.925G>A	p.Gly309Arg
F5P2	F	8	No	4	6	Gastroenteritis, febrile illness	Febrile sz	Hom	c.925G>A	p.Gly309Arg
F6P1	F	15	No	5	6	Infections, gastroenteritis, fasting	No	Hom	c.925G>A	p.Gly309Arg
F7P1	F	14	No	0.9	5	Gastroenteritis, febrile illness, poor eating	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
F8P1	F	11	No	2.5	2.5	Poor food intake	No	Hom	c.925G>A	p.Gly309Arg
F9P1[a]	M	10	No	4	4	Physical activity the previous day	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
F10P1	F	10	Yes	1.5	6	Gastroenteritis, poor food intake	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
F11P1	M	10	No	4	6	Traumatic mouth pain, infections	No	Hom	c.925G>A	p.Gly309Arg
F12P1	M	9	No	4	7	Not known	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
F13P1	M	9	No	1	2	Poor food intake	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg

TABLE 1 (Continued)

Family (F), patient (P)	Gender	Age, years	Neonatal hypoglycemia	Age at first hypoglycemia after neonatal period, years	Age at latest hypoglycemia, years	Triggering factors	Seizure/ hypoglycemic coma	Gene variant zygosity	Nucleotide change	Amino acid change
F14P1	M	6	No	1	5	Infections, poor food intake and physical activity on previous day	Hypoglycemic SE	Hom	c.925G>A	p.Gly309Arg
F15P1	F	7	No	1.5	6.6	Gastroenteritis	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
F16P1	F	3.8	No	1.5	3	Infections, poor food intake	No	Hom	c.925G>A	p.Gly309Arg
F17P1	F	7	No	1.25	3.9	Gastroenteritis, febrile illness, poor eating	Hypoglycemic sz	Hom	c.925G>A	p.Gly309Arg
Average ± SD		11.7 ± 8.8		3.0 ± 2.9	7.4 ± 7.2					

Abbreviations: C/H, compound heterozygote; Hom, homozygote; sz, seizure; SE, status epilepticus.
[a]Published previously.[1]
[b]Dietary precautions since infancy.

Plasma amino acid analysis (Table 3) showed low levels of glucogenic amino acids (especially threonine, serine, proline, glycine, alanine) and urea cycle amino acids (ornithine, citrulline, arginine). In four cases (F2P2, F3P1, F9P1, F11P1), glutamine levels surpassed the upper reference limit (794-1087 μmol/L; normal <709 μmol/L). None of the patients had elevated plasma ammonia. Three patients had low serine (Ser) and glycine (Gly), F13P1, F7P1 and F14P1 (Ser 34, 27, and 37; Gly 66, 65, and 67, respectively, reference range Ser 70-250 μmol/L, Gly 70-350 μmol/L). After complete clinical recovery, the levels of all amino acids normalized, except for one patient with mildly elevated glutamine levels for weeks after an acute hypoglycemia (F3P1).

Urine organic acid profiles raised suspicion for an inborn error of metabolism, showing low or absent ketonuria with prominent fumarate and adipic aciduria in 13/15 PEPCK-C deficient children (Table 4, Figure 2), which is in line with high FFA results and low 3-OHBA results from the blood analysis. The urine organic acid profile was comparable in patients with *PCK1* homozygote and compound heterozygote variants. Lactic aciduria was notable in 12 patients, including the compound heterozygote patient F1P3. The common finding to all PEPCK-C deficient pediatric patients during acute hypoglycemia or fasting test was increased excretion of TCA cycle intermediate fumarate (280 mmol/mol creatinine, normal <20, quantitative measurement from patient F2P2). Excretion of succinate, 2-ketoglutarate and glutarate was more abundant relative to age in many patients, while notable malate and ethylmalonate were observed in fewer cases. Furthermore, high amounts of 5-hydroxyhexanoate, 2-hydroxyadipate and small amounts of rare hydroxyhexenoic acids compounds (3-hydroxyhex-4-enoic acid, 5-hydroxyhex-2-enoic acid) were detected in 5 patients (F3P1, F11P1, F12P1, F13P1, F14P1), similar to those seen in defects of ketogenesis.[25] No pathological acylglycines were found in PEPCK-C deficient patients during acute hypoglycemia or fasting test.

3.3 | Genetic data

Homozygous *PCK1* variant c.925G>A (p.Gly309Arg) (NM_002591.3, GRCh37 g.20:56138747G>A, rs201186470) was detected in 22 patients. The minor allele frequency (MAF) of this variant in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/variant/20-56138747-G-A) is 0.0013. The allele frequency is 26 times higher in Finland (0.012) than in non-Finnish Europeans (0.00046), and there are two homozygous individuals in the control population. In silico predictions of its pathogenicity

TABLE 2 Biochemical parameters in PEPCK-C deficient children during acute hypoglycemia and fasting tolerance test

Biochemical results (Reference range)		PEPCK-C deficient children acute hypoglycemia Mean ± SD (n) (range)	PEPCK-C deficient children fasting test Mean ± SD (n) (range)
B-Glucose mmol/l		0.9 ± 0.4 (n = 16)	2.4 ± 0.6 (n = 11)[a]
(4.2-6.0 mmol/L)		(0.6-2.0)	(1.0-3.2)
B-Lactate mmol/l		4.3 ± 1.8 (n = 15)	3.6 ± 1.0 (n = 9)
(0.33-1.7 mmol/L)		(1.8-9.6)	(1.7-4.8)
3-OHBA mmol/l		1.0 ± 0.6 (n = 7)	0.9 ± 0.4 (n = 8)
(non-fasting < 1 mmol/L)		(0.5-2.3)	(0.6-1.4)
fS-FFA mmol/l		2.5 ± 0.7 (n = 8)	2.6 ± 0,6 (n = 9)
(non-fasting < 0.7 mmol/L)		(1.3-3.5)	(1.4-3.5)
FFA/3-OHBA		2.1 ± 0.7 (n = 5)	3.5 ± 2,0 (n = 8)
		(1.0-3.1)	(1.6-6.9)
P-ALT		68 ± 37 (n = 19)	25 ± 14 (n = 9)[a]
(<50 U/L)		(23-142)	(14-53)
Blood gases from capillary blood	pH (7.35-7.45)	7.33 ± 0.04 (n = 10) (7.26-7.37)	7.36 ± 0.04 (n = 10) (7.29-7.38)
	pCO_2 (4.5-6 kPa)	4.3 ± 0.7 (n = 10) (3.6-5.8)	4.3 ± 0.7 (n = 10) (3.3-5.3)
	HCO_3 (21-28 mmol/L)	16.1 ± 1.9 (n = 10) (14.0-20.0)	17.3 ± 2.1 (n = 10) (15.1-21.0)
	BE (−2.5-2.5 mmol/L)	−9.2 ± 1.9 (n = 10) (−6.2-11.4)	−6.8 ± 1.4 (n = 10) (−4.4-8.9)

[a]$P < 0.001$: PEPCK-C deficient children acute during acute hypoglycemia vs PEPCK-C deficient children during fasting tolerance test.

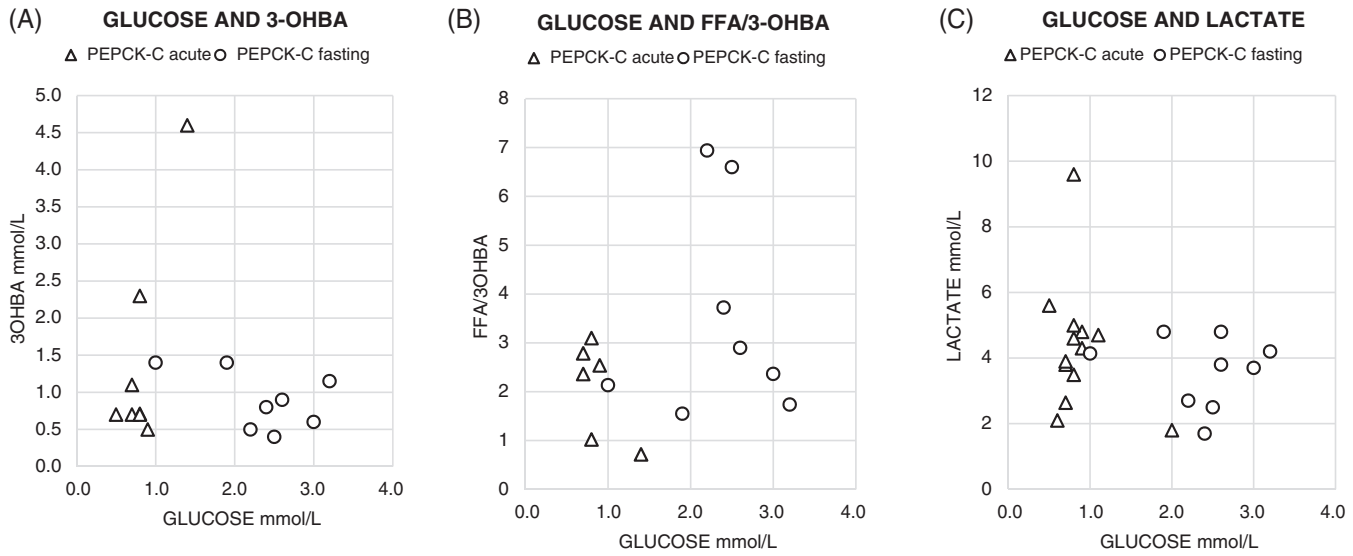

FIGURE 1 B-Glucose, B-3-OHBA, S- FFA/B-3-OHBA, and B-lactate values of PEPCK-C deficient children during acute hypoglycemia and fasting tests. 3-OHBA, 3-hydroxybutyrate; FFA free fatty acids. For n-values, see Table 2

are probably damaging (Polyphen, score 1), deleterious (SIFT, score 0) or disease causing (Mutation Taster, score 0.999), and the CADD score is 26.6 (https://cadd.gs.washington.edu/snv). Previously, it has been shown that in COS-1 cells transfected with this variant PCK1 transcripts were incapable of producing a normally functioning

TABLE 3 Plasma amino acid levels in 8 PEPCK-C deficient children during hypoglycemia (acute and fasting tolerance test). Results are shown as mean ± SD μmol/L

Amino acid	PEPCK-C deficient children	Reference range
Threonine	35 ± 5	34-185
Serine	41 ± 13	60-198
Glutamic acid	30 ± 12	8-83
Glutamine	581 ± 219	304-709
Proline	<50	50-395
Glycine	87 ± 19	74-333
Alanine	192 ± 60	106-522
Citrulline	14 ± 7	6-42
Valine	257 ± 106	85-334
Methionine	9 ± 2	6-29
Isoleucine	81 ± 18	32-119
Leucine	164 ± 80	47-255
Tyrosine	36 ± 3	22-110
Phenylalanine	44 ± 4	21-85
Ornithine	15 ± 4	11-110
Lysine	86 ± 18	57-228
Histidine	52 ± 13	31-99
Tryptophan	<20	20-73
Arginine	19 ± 4	13-98

PEPCK enzyme confirming the pathogenic nature of the variant.[1]

The novel *PCK1* variant c.716C>T (p.Ser239Leu) (NM_002591.3, GRCh37 g.20:56138189C>T, rs139902878) was segregating in family 1 (Table 1, Figure 3). The missense variant p.Ser239Leu has not been reported previously. The MAF of this variant in the Genome Aggregation Database (gnomAD https://gnomad.broadinstitute.org/variant/20-56138189C-T) is 0.000004, CADD score 29.2 (https://cadd.gs.washington.edu/snv) and in silico predictions of its pathogenicity are probably damaging (Polyphen, score 0.935), deleterious (SIFT, score 0) or disease causing (Mutation Taster, score 0.999).

4 | DISCUSSION

A genetic diagnosis of PEPCK-C deficiency was reached for 24 patients during 2016 to 2019. Typical clinical features included morning hypoglycemia after fasting or exercising the day before. Some patients had severe manifestations, such as hypoglycemic coma and status epilepticus, and approximately half of the patients presented with a seizure. Epilepsy was sometimes considered in the differential diagnostics, but eventually all seizures were deemed to have been provoked by hypoglycemia,

TABLE 4 Urine organic acids profiles in 15 PEPCK-C deficient children during hypoglycemia (acute and fasting tolerance test)

Patient	3-OHBA	Lactate	Fumarate[a]	Succinate	2KGA	Malate	EMA	Adipate	Glutarate
F1P3[a]	↑↑↑	↑↑↑	↑↑↑	N	↑↑	↑↑	N	↑↑↑	↑↑
F3P1	↑↑	↑	↑↑↑	↑↑	↑↑↑	ND	↑↑↑	↑↑↑	↑↑
F3P2	↑↑	↑↑↑	↑↑↑	↑↑	↑	ND	N	↑↑↑	↑↑↑
F4P1	↑	↑↑↑	↑↑↑	N/↑	↑	ND	ND	↑↑	↑
F4P2[b]	↑	↑↑↑	↑↑↑	N/↑	↑	ND	ND	↑↑	↑
F5P1[b]	↑	N	↑↑↑	N/↑	ND	ND	ND	↑↑	↑↑
F6P1	ND	N	↑↑	N/↑	ND	ND	ND	↑↑↑	ND
F6P1[b]	↑	↑↑↑	↑↑↑	N/↑	↑↑↑	ND	ND	↑↑	↑↑
F7P1[b]	ND	N	↑↑↑	↑↑↑	↑↑↑	ND	ND	↑↑↑	↑↑↑
F8P1[b]	↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑	↑↑
F9P1	ND	↑↑↑	↑↑↑	ND	↑↑	ND	N	N/↑	ND
F11P1	ND	↑↑↑	↑↑↑	N/↑	↑↑↑	ND	N	↑↑↑	↑↑↑
F12P1	↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	N	N/↑	N/↑
F13P1[b]	↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
F14P1	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑	↑	N	N/↑	↑↑
F17P1	↑↑	↑↑	↑↑↑	N	↑↑	ND	N	↑↑	↑↑

Abbreviations: EMA, ethylmalonate; 2KGA, 2-ketoglutarate; N, normal level; ND, not detected; 3-OHBA, 3-hydroxybutyrate; ↑ increased level.

[a]PCK1-compound heterozygote; all the other patients are PCK1-homozygotes.

[b]Fasting test organic acids profile.

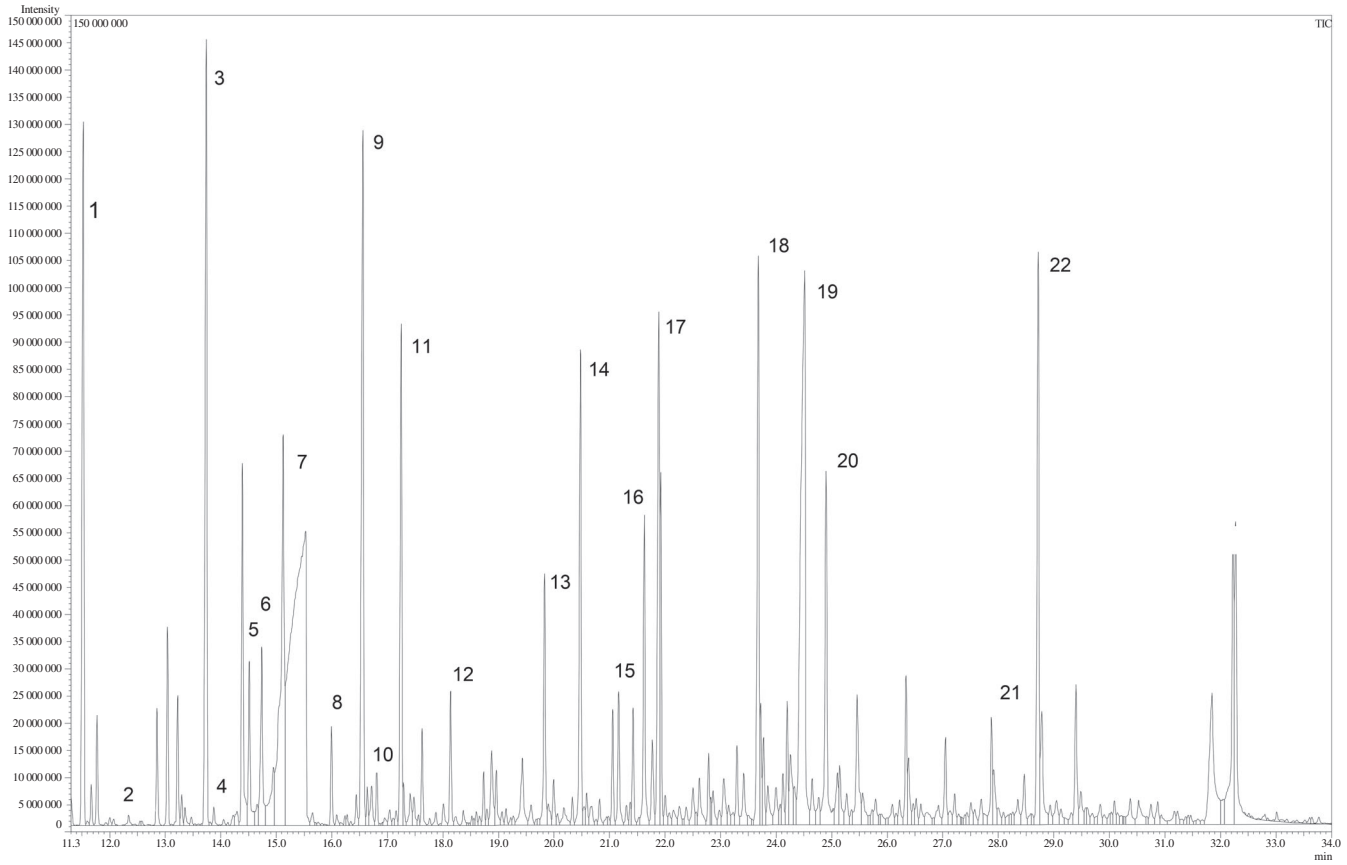

FIGURE 2 Total ion chromatogram of urine organic acids from a PEPCK-C deficient patient (F12P1) during acute hypoglycemia. Abnormal findings are present as prominent lactic aciduria and excretion of TCA intermediates, especially fumaric acid, and as hypoketotic dicarboxylic aciduria (trace amounts of ketone bodies in contrast to large amounts of adipic acid). Metabolite peaks: 1. Lactic acid; 2. 3-methyl-2-oxovaleric acid; 3. 3-hydroxyisobutyric acid coeluting with trace amounts of 3-hydroxybutyric acid; 4. 2-hydroxyisovaleric acid; 5. 2-methyl-3-hydroxybutyric acid; 6. 3-hydroxyisovaleric acid; 7. Ethylhydracrylic acid; 8. Ethylmalonic acid; 9. Succinic acid; 10. Methylsuccinic acid; 11. Fumaric acid; 12. Glutaric acid; 13 Coeluting malic acid and adipic acid; 14. 2-hydroxymethyl-5-furoic acid; 15. 2-hydroxyglutaric acid; 16. 2-ketoglutaric acid; 17. 4-hydroxyphenylacetic acid; 18. Aconitic acid; 19. Hippuric acid; 20. Citric acid; 21. 3-hydroxysebacic acid; 22. Internal standard (margaric acid)

and did not re-occur after the hypoglycemic episodes stopped.

Five patients had neonatal hypoglycemia, but no breast-fed infants presented with hypoglycemia after the neonatal period. This might be because of frequent feedings or other unknown protective factors in that age group. Hypoglycemia could also occur after a regular overnight fast, requiring parental vigilance and adherence to extra carbohydrate intake in the evening, especially if the child consumed less than normal amount of food after a physically active day. A sudden episode of severe gastroenteritis could lead to hypoglycemia even when the underlying disorder was known. However, most of the hypoglycemic episodes were controlled with dietary adjustments after the diagnosis of PEPCK-C deficiency was made. Glucose polymer solution as an ER was well tolerated and easy to administer. The patients had no dietary restrictions, and thus no dietary supplements were considered useful either. One adult patient (F1P2) had several hypoglycemic episodes during breastfeeding, before being aware of the PEPCK-C deficiency. It is possible that the considerable energy loss via breast milk exceeded the tolerance threshold of the PEPCK-C deficient energy metabolism. Lactation does not cause blood glucose fluctuations in healthy normoglycemic women.[26] Another putative risk is alcohol-induced hypoglycemia in adolescents and adults with PEPCK-C deficiency. The oxidation of ethanol to acetaldehyde promotes the reduction of NAD+ to NADH, causing a deficit in NAD+. Decreased availability of NAD+ has been found to affect several metabolic processes, causing decreased gluconeogenesis, glycolysis and Krebs cycle metabolism.[27] NAD+ is required for the cytosolic conversion of malate to OAA, which is then used as the substrate for PEPCK-C. Diminished substrate availability may also impair gluconeogenesis.[28] Thus, individuals with PEPCK-C deficiency may

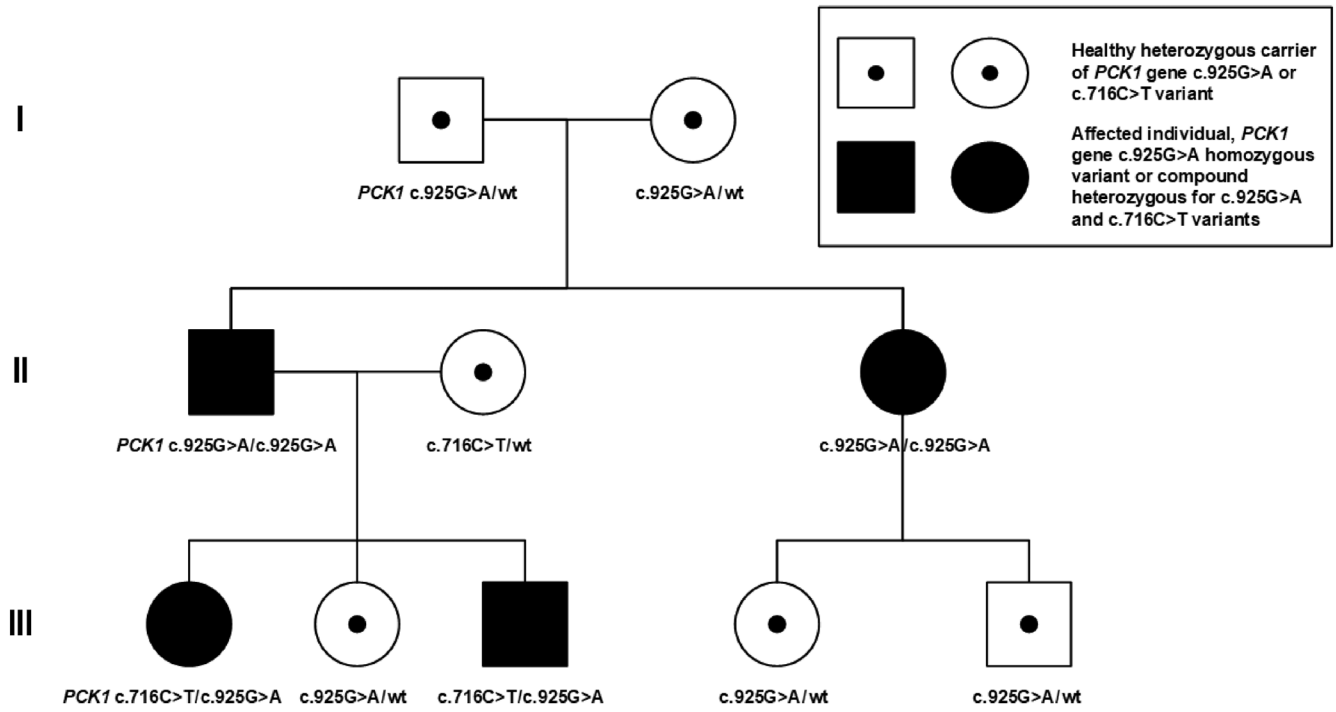

FIGURE 3 Pedigree of Family 1 showing the recessive segregation of the *PCK1* c.925G>A and *PCK1* c.716C>T variants (NM_002591.3)

be susceptible to hypoglycemia after consuming alcohol in situations that would otherwise be tolerated.

Characteristic laboratory findings included severe hypoglycemia, relatively low ketone levels, elevated liver transaminase values and abnormal urine organic acids profiles, especially fumarate being prominently excreted. Most of the PEPCK-C patients had hypoketotic hypoglycemia, but PEPCK-C deficiency cannot be excluded by a normal ketone response, as shown by patient F15P1. Extensive biochemical testing was performed for many patients in this study before the correct diagnosis was established. The high FFA/3-OHBA ratio during acute hypoglycemia was comparable to the typical ratio seen in fatty acid oxidation disorders,[22,29] and this was a common working hypothesis in the initial clinical investigation. In the liver-specific PEPCK-C knockout mouse model, relatively low blood ketone levels and high FFA levels were associated with hepatic steatosis.[9] One patient (F3P1) had persistent fatty liver ultrasound finding for several months after a severe hypoglycemic episode. The patients in this PEPCK-C deficiency cohort had high circulating FFA levels indicating normal activation of lipolysis and mobilization of FFA from fat storages. Low ketone production relative to FFA suggests deficient ketogenesis. Interestingly, urine organic acids profiles included abnormal rare compounds (3-hydroxyhex-4-enoic acid, 5-hydroxyhex-2-enoic acid) described in defective ketogenesis,[25] providing further evidence of insufficient ketone production. These compounds have been suggested to form six-carbon-or-longer fatty acids, which are metabolized via secondary pathways under conditions of deficient ketone production in hypoketotic hypoglycemia.[25] Adequate ketone production during prolonged fasting is especially important in young children,[22,29] who are more prone to hypoglycemia due to smaller liver glycogen stores.[21]

The urine organic acid profile of PEPCK-C deficient patients was comparable during acute hypoglycemia and fasting tolerance test, showing a recognizable pattern of low ketonuria, prominent adipic aciduria, prominent fumaric aciduria and in most cases prominent lactic aciduria. The urine fumarate measured in patient F2P2 was comparable to levels described in fumarase deficiency.[30] It has also been reported previously that plasma glutamine is elevated in PEPCK-C deficient patients,[12] linked to proximal urea cycle dysfunction. In this study, mildly elevated plasma glutamine was seen in only four patients. This could possibly be associated with a milder phenotype as plasma ammonia levels were not elevated in this cohort. Some patients exhibited transiently low plasma serine and glycine values, suggesting an increased consumption of these glucogenic amino acids.[31] It remains unclear whether the low levels of these amino acids contributed to the acute neurological symptoms. Cerebral spinal fluid serine or glycine values were not available for any of the patients in this study, which limits our ability to draw conclusions on this.

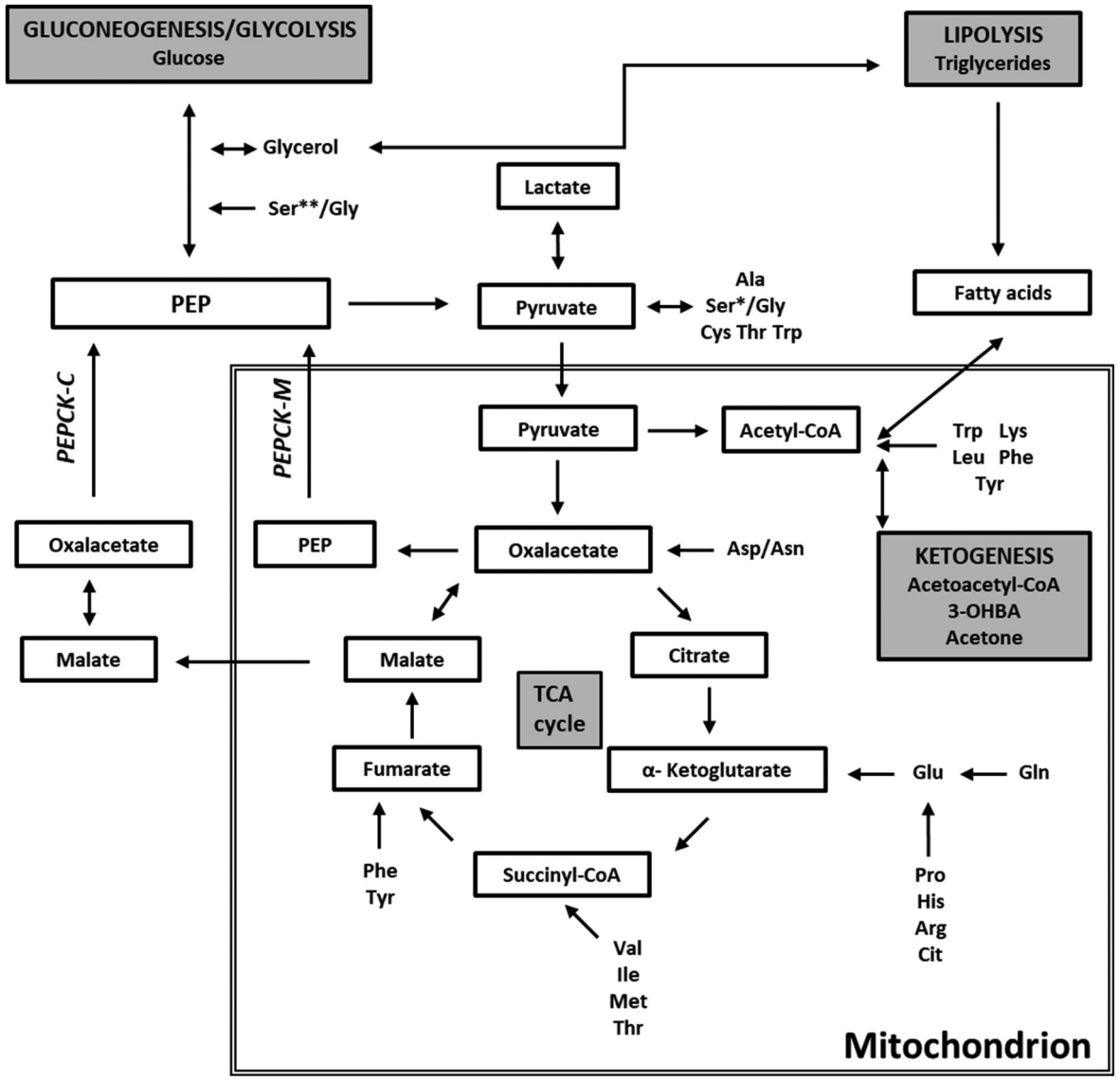

FIGURE 4 Scheme of metabolic pathways and metabolites related to PEPCK-C. * Gluconeogenesis via SDH pathway, ** gluconeogenesis via SPT/AGT. Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cit, citrulline; Cys, cysteine; Glu, glutamate; Glm, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Lys, lysine; Leu, leucine; Met, methionine; Phe, phenylalanine; Pro, proline; PEP, phosphoenolpyruvate; SDH, serine dehydratase; Ser, serine; SPT/AGT, pyruvate/alanine:glyoxylate aminotransferase; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine

Based on the biochemical data, the correlation between the blood glucose levels and the counter regulatory energy metabolism response seemed to be impaired, suggesting that patients with PEPCK-C deficiency might have a more complex energy metabolism disorder, beyond the disturbance in gluconeogenesis (Figure 4). PEPCK-C flux has been suggested to have a role in the coordination of hepatic energy metabolism but not as a sole controller of the gluconeogenesis rate.[10] There may, however, be other unknown factors involved.

PCK1 variant c.925G>A is enriched in the Finnish population, and its MAF in healthy Finnish controls (0.012) is equivalent to those estimated for the so-called Finnish heritage diseases.[32] Finland's recent population bottleneck and genetic drift has led to the enrichment of rare recessive variants in the population and the identification of pathogenic founder variants explaining several recessive diseases in Finland.[32,33] The enrichment of *PCK1* c.925G>A variant allows the use of targeted Sanger sequencing approach of this known variant in Finnish patients suspected to have *PCK1* gene related hypoglycemia. The presence of two homozygous individuals in healthy controls indicates that at least some individuals may have mild undiagnosed clinical presentation or later onset of the disease. The novel variant c.716C>T (p.Ser239Leu) is proposed to be likely pathogenic according to the published ACMG guidelines,[34] based on the segregation data, computational evidence for

deleterious effect, the extremely low presence in control population, and detection in trans with a known pathogenic variant. The clinical phenotype of childhood-onset symptomatic hypoglycemia, and the urine organic acid profile were similar to the patients with homozygous c.925G>A(p.Gly309Arg) variant of *PCK1*. The enzymatic analysis as a further proof of pathogenicity was not available for the PEPCK-C protein harboring the novel p.Ser239Leu variant, but the PEPCK-C protein with homozygous p.Gly309Arg has previously been shown to possess extremely low PEPCK-C enzyme activity, comparable to the empty vector in transfected COS-1 cells.[1]

This study implies that PEPCK-C deficiency might be a more common cause of unexplained childhood hypoglycemia than previously thought, at least in the Finnish population, suggesting that PEPCK-C deficiency could be a new candidate for the Finnish disease heritage.

5 | CONCLUSIONS

The genetic etiology of recurrent hypoglycemia is highly heterogeneous. The identification of the genetic cause allows for correct therapeutic and nutritional advice. Cytosolic PEPCK deficiency poses a treatable inborn error of metabolism, which may be more common than previously thought. The laboratory findings include deficient ketogenesis and a recognizable pattern of urine organic acids. The tendency to develop hypoglycemia is predominant but not limited to young age groups. The timely correct diagnosis and patient guidance is important also for adults. Our study expands the spectrum of this metabolic disorder to a lifelong disease.

CONFLICT OF INTEREST

Päivi Vieira, Irina I. Nagy, Marja-Leena Väisänen, Katariina Latva, Pekka Valmari, Virpi Sidoroff, Riikka Keski-Filppula, Kari Kaunisto, Maria K. Haanpää, Päivi J. Miettinen, Marja Ojaniemi, Matti Nuutinen, Tuula Arkkola and Päivi Myllynen declare that they have no conflict of interest. Elisa Rahikkala has received research grants from the Maija and Matti Vaskio Fund of the Finnish Medical Foundation, the Ester and Uuno Kokki Fund of the Finnish Cultural Foundation and the Academy of Finland (decision number 338446). Johanna Uusimaa has received a research grant from the Academy of Finland (decision number 331436).

AUTHOR CONTRIBUTIONS

Päivi Vieira: Study design, acquisition, analysis, interpretation of data, first draft of the manuscript and Table 1, critical review; **Irina I. Nagy**: Study design, acquisition, analysis, interpretation of data, first draft of the Tables 2-4, Figures 1, 2 and 4 in addition to parts of the manuscript, critical review; **Elisa Rahikkala**: Acquisition, analysis, interpretation of data, drafting parts of the manuscript, final version of Figure 3, critical review; **Marja-Leena Väisänen**: Acquisition, analysis, interpretation of data, drafting parts of the manuscript, critical review; **Katariina Latva**: Acquisition, analysis of data, critical review; **Pekka Valmari**: Acquisition, analysis, interpretation of data, first draft of Figure 3, critical review;: **Virpi Sidoroff**: Acquisition of data, critical review; **Riikka Keski-Filppula, Kari Kaunisto, Maria K. Haanpää, Päivi J. Miettinen, Marja Ojaniemi, Matti Nuutinen**: Acquisition, analysis, interpretation of data, critical review; **Tuula Arkkola**: First draft of Table S1, acquisition and interpretation of nutritional data, critical review, **Johanna Uusimaa**: Study design, analysis, interpretation of data, critical review; **Päivi Myllynen**: Study design, acquisition, analysis, interpretation of data, critical review.

DATA AVAILABILITY STATEMENT

The novel variant segregating in family 1 was submitted to the LOVD database (https://databases.lovd.nl/shared/genes/PCK1) hosted at Leiden University Medical Center, the Netherlands. Patients' medical records are confidential. Other data supporting the findings are available from the authors upon reasonable request.

ETHICS STATEMENT

The study was approved by the Regional Ethics Committee of the Northern Ostrobothnia Hospital District. The guidelines of the Helsinki Declaration were followed.

PATIENT CONSENT STATEMENT

A written informed consent was obtained from the study subjects or their guardians.

ORCID

Päivi Vieira https://orcid.org/0000-0002-0796-8706
Päivi J. Miettinen https://orcid.org/0000-0002-5184-9616
Johanna Uusimaa https://orcid.org/0000-0002-6794-209X

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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