Molecular Genetics and Metabolism 118 (2016) 21–27

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

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

Cytosolic phosphoenolpyruvate carboxykinase deficiency presenting with acute liver failure following gastroenteritis

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ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 3 March 2016
Accepted 3 March 2016
Available online 4 March 2016

Keywords:
PEPCK
PCK1
Hyperammonaemia
Hepatopathy
Lactic acidosis
Treatment

ABSTRACT

We report a patient from a consanguineous family who presented with transient acute liver failure and biochemical patterns suggestive of disturbed urea cycle and mitochondrial function, for whom conventional genetic and metabolic investigations for acute liver failure failed to yield a diagnosis. Whole exome sequencing revealed a homozygous 12-bp deletion in *PCK1* (MIM 614168) encoding cytosolic phosphoenolpyruvate carboxykinase (PEPCK); enzymatic studies subsequently confirmed its pathogenic nature. We propose that PEPCK deficiency should be considered in the young child with unexplained liver failure, especially where there are marked, accumulations of TCA cycle metabolites on urine organic acid analysis and/or an amino acid profile with hyperammonaemia suggestive of a proximal urea cycle defect during the acute episode. If suspected, intravenous administration of dextrose should be initiated. Long-term management comprising avoidance of fasting with the provision of a glucose polymer emergency regimen for illness management may be sufficient to prevent future episodes of liver failure. This case report provides further insights into the (patho-)physiology of energy metabolism, confirming the power of genomic analysis of unexplained biochemical phenotypes.

1. Introduction

The availability of whole exome sequencing (WES) has revolutionized the diagnosis of patients with inborn errors of metabolism (IEM), who remain undiagnosed following conventional biochemical and genetic testing [1]. The explosion in reports of novel IEM in recent years is testimony to this [2]. WES can also expand the clinical phenotype of known IEM by the discovery of variants in patients investigated for clinical presentations not previously recognised to be associated with that diagnosis [3]. In these situations, ascribing pathogenicity to such variants may require more detailed functional investigations and in vitro experiments in order to confirm that the detected variants are responsible for the observed presentation. We report a patient from a consanguineous family who presented with transient acute liver failure and biochemical patterns suggestive of disturbed urea cycle and mitochondrial function, for whom conventional genetic and metabolic investigations for acute liver failure failed to yield a diagnosis. WES performed through the Omics2TreatID study revealed a homozygous 12-bp deletion in *PCK1* (MIM 614168) encoding cytosolic phosphoenolpyruvate carboxykinase (PEPCK) as well as a hemizygous variant in *PHKA2* (MIM 300798), one of the genes encoding the alpha subunit of glycogen phosphorylase kinase and associated with X-linked glycogen storage disease type IX. Detailed enzymatic studies have subsequently confirmed the pathogenic nature of the *PCK1* deletion. PEPCK, an important regulatory step in gluconeogenesis, has only rarely previously been implicated in disease and most historical cases have relied solely upon enzymatic confirmation of the diagnosis. This is difficult due to there being two discrete subcellular isoforms of PEPCK: cytosolic PEPCK (encoded by *PCK1*) and mitochondrial PEPCK (encoded by *PCK2*). Total PEPCK activity can also be decreased as a secondary phenomenon. Confirmation of a pathogenic *PCK1* variant makes this only the second report in the literature of genetically confirmed cytosolic PEPCK

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http://dx.doi.org/10.1016/j.ymgme.2016.03.001
1096-7192/

deficiency and suggests this IEM might be an under-recognised cause of transient acute liver failure in childhood.

2. Methods

2.1. Ethics

The Omics2TreatID study was approved by the BC Children's & Women's Hospital and University of British Columbia Ethics Board (Vancouver, Canada (H12-00067)). Parents provided informed consent for publication of this report.

2.2. NGS sequencing

Genomic DNA was isolated from the peripheral blood of the patient, and unaffected parents using standard techniques. WES was performed for all three family members using the Agilent V4 51Mb with Illumina HiSEQ 2000 100 bp pair-end reads. An in-house designed bioinformatics pipeline [4] comprising of Bowtie2 [5], Genome Analysis Toolkit [6], SnpEff [7] and SAMtools [8], and was used to align the reads to human reference genome version hg19 and to identify and assess rare variants for their potential to disrupt protein function. The average coverage across the known coding exons was 42×. Rare variants were identified based on a comparison against allelic frequencies from dbSNPv142, Exome Variant Server, Exome Aggregation Consortium (ExAC) and an in-house database of more than 430 exomes and whole-genomes using minor allele frequency (MAF) 1% as the allelic threshold. The remaining variants were subsequently screened under a series of genetic models (e.g. homozygous recessive, hemizygous recessive, compound heterozygous, de novo heterozygous). Within each inheritance model, mutations were further prioritized according to putative impact at the variant level (e.g. CADD score) [9], and at the gene level (e.g. RVIS score) [10].

2.3. Sanger sequencing

Sanger sequencing was performed according to standard methods in all family members to confirm segregation with disease of both the *PCK1* deletion and *PHKA2* variant.

2.4. PCK1 mutagenesis and transfection into COS-1 cells

Human cytosolic phosphoenolpyruvate carboxykinase 1 (*PCK1*) full-length cDNA (GenBank accession # NM_002591) was cloned in pcDNA3.1 mammalian expression vector (GenScript). The mutant *PCK1* construct with 12 base pair nucleotide deletion was created by synthesis of the nucleotide fragment and subcloning. COS-1 cells were transfected with pcDNA3.1 plasmids using lipofectamine 2000 reagent (Life Technologies) for 48 h. The whole cell lysates were used in PCK1 enzyme activity assay and for Western Blot analysis.

2.5. PEPCK Western blot studies

For both liver biopsy and transfected COS-1 cells, Western blotting was performed for analysis of PCK1 protein expression. 10–25 μg protein was fractionated by electrophoresis in SDS-polyacrylamide gel, transferred to PVDF membrane, and then immunoblotted with polyclonal anti-PCK1 antibody (Proteintech; 16754-1-AP) with 1:1000 dilution. GAPDH was used as a control antibody.

2.6. PEPCK-C enzyme activity

PEPCK catalyzes the reversible GTP-dependent decarboxylation of oxaloacetate. PEPCK activity was measured in the direction of oxaloacetate formation using published methods [11–13]. The reaction follows the GDP and Mn^{2+}-dependent carboxylation of phosphoenolpyruvate. 0.05% Triton X-100 was used to disrupt the cells and mitochondria, and NADH and malate dehydrogenase were included to ensure unstable oxaloacetate was converted to malate. The incorporation of [^{14}C] from 1-^{14}C-$NaHCO_3$ into oxaloacetate and subsequently malate, was used to calculate PEPCK activity. ADP was used instead of GDP as a blank. Approximately 2×10^5 COS-1 cells were trypsinized, washed twice in PBS and resuspended in 100 μL 1 mM dithiothreitol. The cells were disrupted by freeze-thawing in liquid nitrogen before use. For tissue, liver (frozen in liquid nitrogen, and stored at −80°), was homogenized in ~7× w/v buffer (0.25 M sucrose, 0.01 M Tris, 0.001 M EDTA pH 7.4), using a glass Wheaton homogenizer. The homogenate was centrifuged in an Eppendorf centrifuge at 16 K for 25 min. The supernatant was saved as the cytosolic fraction. The pellet was then resuspended in lysis buffer (5 mM KPi, pH 7.4, 1 mM EDTA, 0.1 mM DTT), rehomogenized as before and centrifuged for 10 s. The supernatant was saved as the mitochondrial fraction. All reactions were performed in duplicate.

3. Results

3.1. Case report

The index case was a nine-month old boy, the fifth child born at term after an uneventful pregnancy and delivery to third-cousin consanguineous parents of Pakistani origin whose elder four children were healthy with normal psychomotor development (Fig. 1).

He presented, after an otherwise uneventful early infancy with normal somatic growth and acquisition of developmental milestones, during an episode of gastroenteritis. After three days of persistent diarrhoea and vomiting, he was taken to hospital with symptoms of encephalopathy and investigations revealed a mild hyperammonaemia (146 μmol/L) and evidence of acute liver failure, with very elevated alanine transaminase (maximum: 10003 IU/L [reference range: 5–45]), aspartate transaminase (maximum: 9582 IU/L [reference range: 0–80]), coagulopathy (maximum prothrombin time: 15 s [reference range: 9–13] and hypoalbuminaemia (minimum: 29 g/L [reference range: 34–42]). Mild hypoglycaemia (2.8 mmol/L [reference range: 3.5–6.0]) and hyperlactataemia (maximum: 2.9 mmol/L [reference range: 0.6–2.5]) were also recorded.

Following transfer to our tertiary paediatric liver unit, conservative management for acute liver failure was commenced with intravenous dextrose-containing fluids, piperacillin/tazobactam, fluconazole, acyclovir and *N*-acetylcysteine. A rapid normalization of his level of consciousness after commencement of intravenous dextrose was noted, along with more gradual improvement in his liver failure over the course of the following week. During this admission, initial metabolic investigations for neonatal liver failure excluded classical galactosaemia, hereditary tyrosinaemia type 1, alpha-1-antitrypsin deficiency and fatty acid oxidation disorders (with an acylcarnitine profile indicative only of ketosis). Plasma quantitative amino acid analysis was suggestive of a proximal urea cycle defect (UCD) with a strongly elevated glutamine (1731 μmol/L [reference range: 333–809]) together with low citrulline (5 μmol/L [reference range: 8–47]) and arginine (10 μmol/L [reference range: 12–112]). Furthermore analysis of urine organic acids by gas chromatography/mass spectrometry on two occasions, three days apart, during the presenting illness demonstrated prominent tricarboxylic acid (TCA) cycle metabolites: in particular fumarate but also succinate, malate and alpha-ketoglutarate. 3-hydroxybutyrate and acetoacetate were slightly increased as were several dicarboxylic acids and hydroxy-dicarboxylic acids, particularly adipate, 3-hydroxysebacate and 3-hydroxydodecanedioate; the latter were felt possibly secondary to ketosis. Orotic acid was slightly increased in the second specimen (5.4 μmol/mmol creatinine [reference range < 3]). Phenolic acids often associated with liver failure were not increased. Glycerol was not increased.

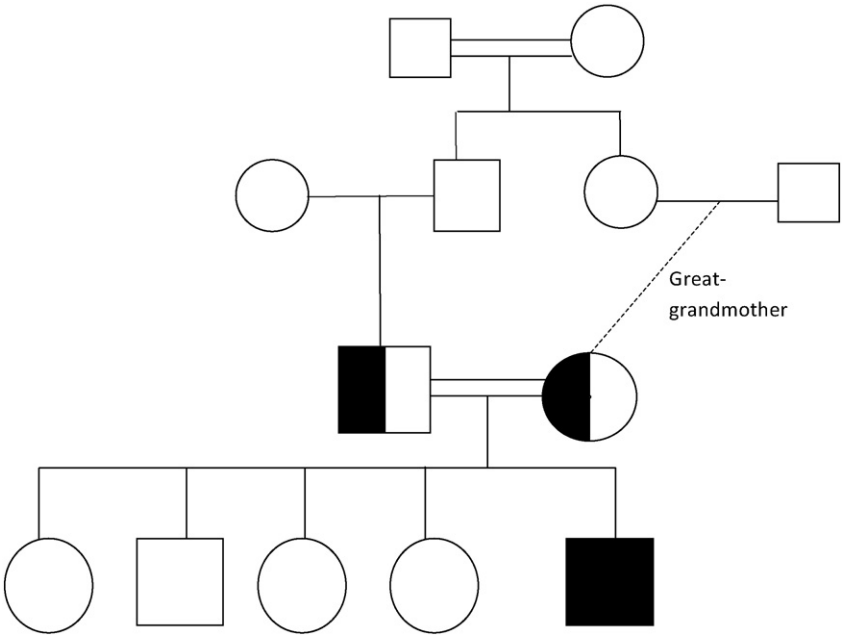

Fig. 1. Family pedigree indicating distant consanguinity.

Liver biopsy during this illness demonstrated diffuse macrosteatosis without fibrosis or increase in inflammatory cell infiltrate; ultrasound showed a normal-sized liver for age, but with hyperechoic parenchyma.

Whilst diagnostic investigations were pending, the child was commenced on treatment for a proximal UCD with sodium benzoate, sodium phenylbutyrate and L-arginine as well as dietary protein restriction to 1.5 g/kg/day natural protein.

Diagnostic investigations for both UCD and mitochondrial disorders were arranged in parallel (Table 1) and failed to confirm an underlying IEM in either of these areas (Table 1). Molecular analysis of *CA5A* (MIM 114761) to rule out a novel IEM associated with a combined proximal UCD and mitochondrial abnormalities, carbonic anhydrase VA deficiency (MIM 615751) [2,14] as well as of *CA5B* (MIM 300350) - an X-linked gene with similar function but not yet associated with human disease - were both negative.

Repeat organic acid and amino acid analysis a month later when the child had completely recovered showed no evidence of the previous abnormalities and have remained normal since the subsequent withdrawal of UCD medications and return to an unrestricted diet.

3.2. WES results

Due to the severe clinical presentation triggered by illness and the remaining diagnostic uncertainty despite significant metabolic abnormalities at the time of illness, the patient was enrolled into the Omics2TreatID study for trio WES analysis, which was performed as described in the Methods section [15]. Four candidate genes were identified from homozygous or hemizygous models, 5 compound heterozygous candidate genes and 5 candidate genes from a de novo heterozygous model (Supplementary Table 1). These variants represented rare coding variants that passed good alignment thresholds by manual screening on Integrative Genomics Viewer [16], and are predicted to be damaging with collective agreement from CADD (normalized score ≥ 15), SIFT and PolyphenV2.

Given the parental consanguinity, a recessive mode of inheritance was assumed, but all 15 genes harbouring rare non-synonymous or splice-site variants were carefully reviewed for a possible relation with the observed phenotype. The best fits included a novel, homozygous 12 bp deletion in *PCK1* as well as a non-synonymous single base change in *PHKA2*. The functional/disease-relevance summaries for the remaining genes and their respective OMIM references provided in the supplementary table show how none of the other candidates came close to explain the patient phenotype and were therefore ruled out as unlikely.

The 12 bp homozygous deletion "GTGTCCCTCTAG" located on chromosome 20:56140097-56140107 (hg19) affecting *PCK1* was considered a very strong candidate because of the likely damaging effect of a four amino-acid deletion (p.GVPLV123V), as well as being an enzyme with known expression in liver and its involvement in the body's response to starvation for this child who decompensated with liver failure after a period of prolonged fasting. The deleted amino acids were all highly conserved from human to zebrafish according to the 46-way

Table 1
Results of metabolic investigations into urea cycle and mitochondrial disorders.

Urea cycle disorders		Mitochondrial disorders			
CPS deficiency	No pathogenic variants identified in *CPS1* cDNA from cultured leucocytes	Single gene disorders	No pathogenic variants identified in *POLG*, *DGUOK*, *MPV17* or *TRMU*		
NAGS deficiency	No pathogenic variants identified in *NAGS*	Respiratory chain enzymes (muscle)	Normal results (activities expressed as a ratio to citrate synthase)		
			Complex I	0.178	0.104–0.268
			Complex IV	0.017	0.014–0.034
			Combined Complex II + III	0.071	0.040–0.204
Carbonic anhydrase deficiency	No pathogenic variants identified in *CA5A and CA5B*	mtDNA mutations	No evidence of m.8993, m.3243 and m.8344 mutations		

Conservation track in UCSC Genome Browser. The deletion falls within the N-terminal of phosphoenolpyruvate carboxykinase domain and adjacent to a putative GTP-binding domain [17]. Sanger sequencing confirmed segregation of this variant with disease as expected for a homozygous recessive model (Fig. 2). The variant has not been previously reported in dbSNP (version 142), nor in NHLBI ESP, our in-house genome database comprising of over 430 exomes and whole-genomes, and 61,486 unrelated individuals from ExAC (accessed August 04, 2015). The variant is predicted to affect splicing by MutationTaster [18] and marked to be damaging by SIFT INDEL [19].

An X-linked hemizygous non-synonymous variant in *PHKA2* (chrX: 18912416; T1148M) was also selected because of the relevance of the liver phosphorylase kinase enzyme in the hepatic response to prolonged starvation. The mutation has a CADD score of 18.31 and is predicted damaging by SIFT, PolyPhenV2 and MutationTaster. Sanger sequencing confirmed this variant and testing of family members showed segregation of this variant as expected for an X-linked recessive model, with the unaffected mother as carrier.

3.3. Diagnosis of cytosolic PEPCK deficiency

The small amount of homogenized liver tissue remaining from the child's initial investigations was subjected to a Western Blot with anti PCK1 antibody, which demonstrated decreased PCK1 levels in the patient's liver compared to a control liver sample from a three month old boy, but this was not deemed statistically significant. (Fig. 3-A).

A second liver biopsy was performed under general anaesthesia, but with intravenous dextrose administration during the preoperative fast, at the age of three years and analysed for PEPCK enzyme activity in the cytosolic and mitochondrial fractions. Interestingly there was no evidence of histological steatosis on this sample, which was taken during a period of metabolic stability. Compared to a control human liver sample analysed in parallel, which showed a PEPCK activity in the cytosolic fraction approximately two times higher than in the mitochondrial fraction, this ratio was reversed in the patient's liver sample showing a mitochondrial activity nearly a third higher than in the cytosolic fraction (Table 2). Analysis of Complex IV activity confirmed appropriate subcellular fractionation.

To further investigate the pathogenicity of this deletion, *PCK1* was overexpressed in COS-1 cells. Western blotting showed that COS-1 cells transfected with empty vector had no PCK1 protein expression (Fig 3-B). Transfection of wildtype *PCK1* produced an increased amount of PCK1 protein expression in COS-1 cells, and transfection of the *PCK1*-deletion mutant produced expression comparable to the patient's ex-vivo liver cells (Fig 3-B). Measurement of total PEPCK enzyme activity showed the following results: COS-1 cells have extremely low endogenous PEPCK activity (mitochondrial and cytosolic), and negligible PEPCK activity was also seen in empty vector. Compared to wildtype transfected COS-1cells, mutant PEPCK activity was significantly reduced, similar to the empty vector. These results confirmed the deleterious nature of the homozygous *PCK1* deletion mutant, abolishing PEPCK enzyme activity (Table 3, Fig 3-C).

3.4. Exclusion of glycogen storage disease type IX

A diagnosis of glycogen storage disease type IX (due to a *PHKA2* mutation) had not been considered at the outset as this is not usually associated with infantile liver failure. However the WES results prompted us to further investigate the patient for this IEM. Clinically the child did not have hepatomegaly at presentation and the ultrasound appearance of the liver was normal at three years of age, when the child was admitted for overnight glucose monitoring and no nocturnal hypoglycaemia was noted during 10 h of fasting. Erythrocyte glycogen content was normal (74 μg/g Hb [reference range: 10–120]) as was erythrocyte phosphorylase b kinase activity (15 μmol/min/g Hb [reference range: 10–90]). Surrogate markers of glycogen storage disease such as plasma uric acid, cholesterol and triglycerides as well as urine oligosaccharides were normal. The liver sample collected at three years of age demonstrated no

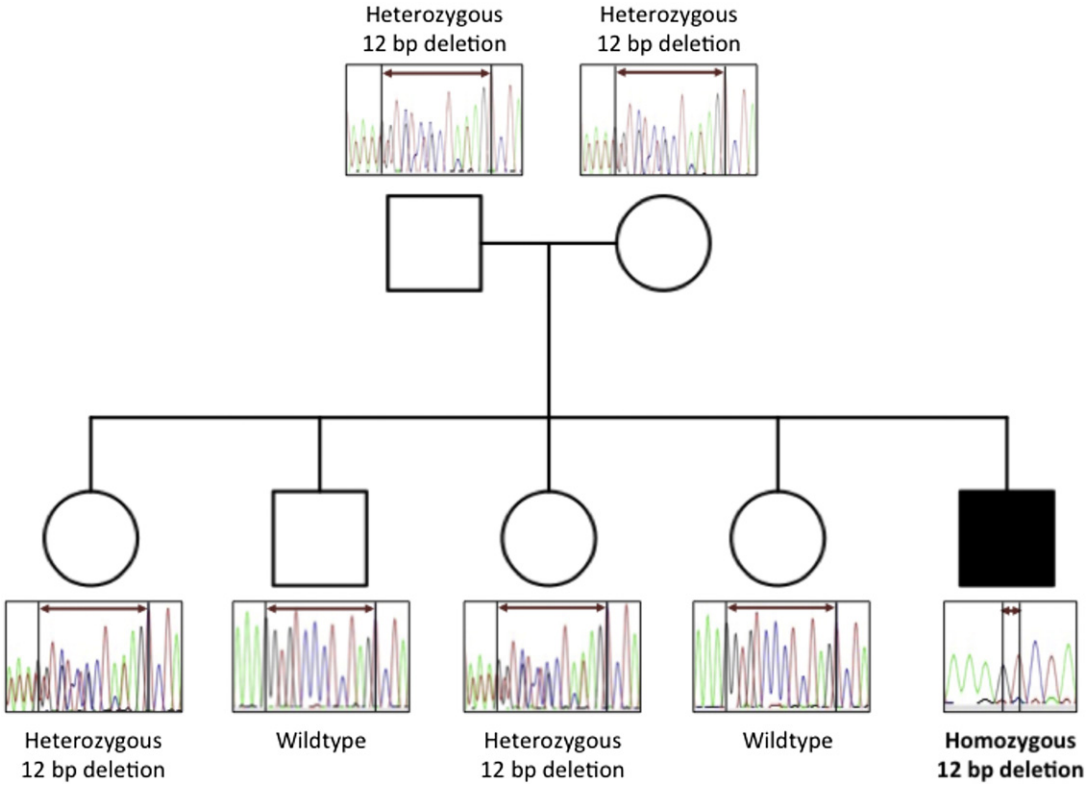

Fig. 2. Segregation of 12 bp deletion in *PCK1* in family consistent with a homozygous recessive inheritance pattern.

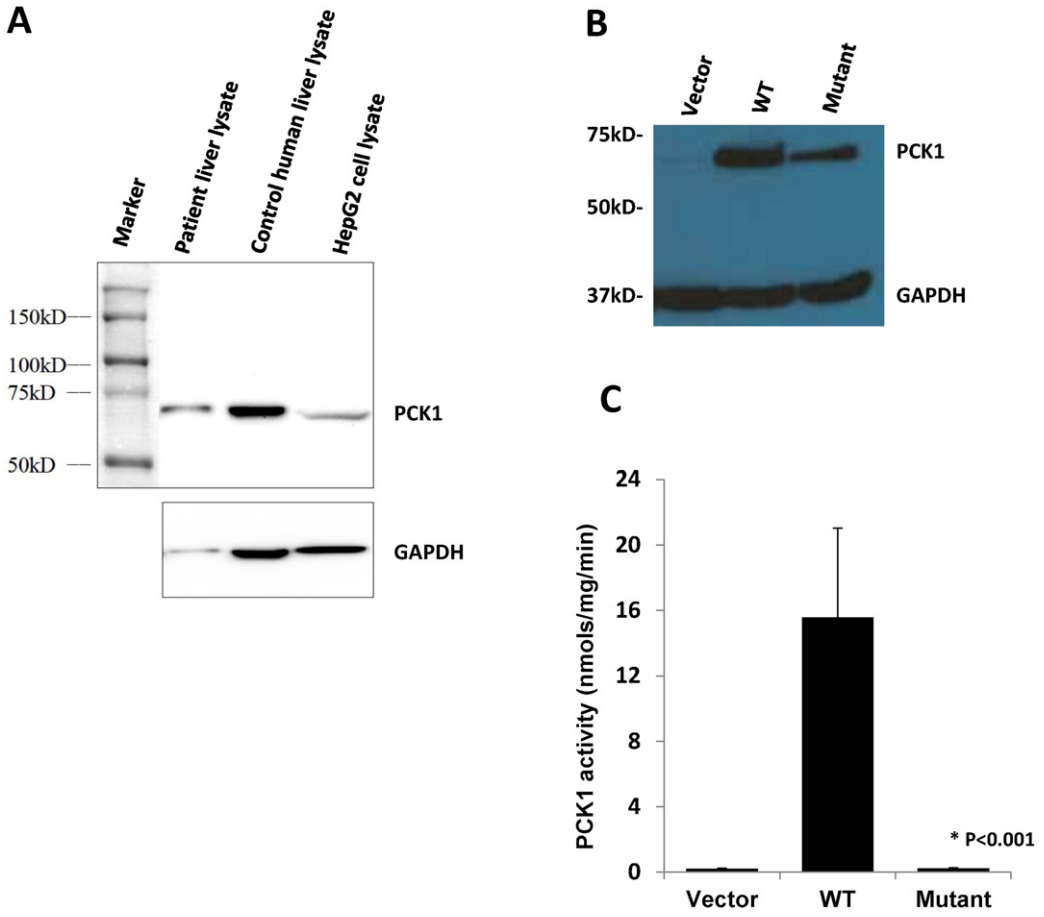

Fig. 3. Functional analysis of PCK1 mutant. (A) Western analysis of patient liver biopsy sample; (B) PCK1 protein expression in transfected cells; (C) PEPCK enzyme activity in transfected cells. Vector: pcDNA3.1 empty vector; WT: wild type PCK1; Mutant: PCK1 with 12-bp deletion. *$p < 0.001$: mutant vs. wild type PCK1 (mean ± SD; n = 3–4).

significant histological glycogen storage and showed phosphorylase b kinase activity of 5742 μmol/min/g protein which was comparable to simultaneous normal controls of 4378 and 3934 μmol/min/g protein, thereby excluding co-existing glycogen storage disease type IX as a contributory factor to this child's presentation.

3.5. Clinical course

Even after the child's dietary protein restriction was relaxed, a glucose polymer emergency regimen was maintained and updated according to the child's age. Now aged four years, he has had no further episodes of liver failure, though has not suffered any further episode of gastrointestinal infection and has not been allowed to fast for a prolonged period. Linear growth has maintained along the 50th percentile and weight between 91st and 98th percentile. Development appears to be within normal limits for his age. The steatosis observed in the patient's liver during illness is in keeping with the findings in the few other described PCK1 deficiency patients [20–22].

4. Discussion

Clinical descriptions of enzymatically confirmed PEPCK deficiency were first reported in the 1970s by three groups reporting a total of 4 children: two individual cases and two siblings suffering from hypoglycaemia and lactic acidosis [20–22]. Where performed, histological evidence of hepatic steatosis was present. Furthermore a case of mitochondrial PEPCK deficiency was reported in a three month old British girl who had fatal liver failure, encephalopathy and hypoglycaemia [23] although investigation of a subsequent sibling was not suggestive of PEPCK deficiency [24] and a mitochondrial DNA depletion disorder was proposed as the underlying cause [25]. A Japanese case has been described with hypoglycaemia and fatty change in the liver but only with enzymatic PEPCK deficiency and without subcellular differentiation (cytosolic or mitochondrial) [26]. In these historical cases with only enzymatic PEPCK measurement, the diagnosis of PEPCK deficiency cannot be relied upon as this may be a secondary phenomenon and accurate subcellular differentiation was not always recorded. Until now, and only recently, two patients have been reported with clinically relevant *PCK1* mutations and these two siblings presented with episodic fasting

Table 2
PEPCK and Complex IV enzyme activity is shown for control human and patient liver samples. Standard error is shown, and number of replicates is in parentheses.

Experiment		Patient	Control human liver
Complex IV activity (nmol/mg protein/min)	Cytosol (C)	1.36 (1)	0.34 (1)
	Mitochondria (M)	41.05 (1)	17.9 ± 0.6 (2)
	C:M ratio	1:30	1:53
PEPCK activity (nmol/mg protein/min)	Cytosol (C)	20.86 (1)	7.86 (1)
	Mitochondria (M)	27.71 (1)	3.73 (1)
	C:M ratio	0.75:1	2.1:1

Table 3
PEPCK enzyme activity is shown for COS-1 cells transfected with empty vector, wildtype *PCK1* and deletion mutant *PCK1* genes. Enzyme activity is shown ± standard error, with the number of replicates in parentheses.

COS-1 transfection experiment	PEPCK activity (nmol/mg protein/min)
COS-1	0.11 ± 0.01 (3)
COS-1 + empty vector	0.21 ± 0.03 (4)
COS-1 + wildtype *PCK1*	15.57 ± 5.46 (4)
COS-1 + *PCK1* (12 bp deletion)	0.23 ± 0.04 (4)

hypoglycaemia and lactic acidosis in addition to other genetic syndromic diagnoses [27].

PEPCK is one of the first steps of gluconeogenesis and in vivo is believed to catalyze the conversion of oxaloacetate into phosphoenolpyruvate and carbon dioxide (Fig. 4) [28].

PEPCK is an important regulatory step of gluconeogenesis, with transcription of *PCK1* stimulated by glucagon, cortisol and catecholamines in response to fasting and repressed by insulin in the post-prandial state [29,30]. Unlike in the more common gluconeogenic disorder, fructose-1,6-bisphosphatase deficiency, gluconeogenesis from glycerol is unimpaired and therefore glyceroluria (strongly suggestive of a gluconeogenic disorder) is expected to be absent. In the regularly fed state, therefore, one can expect that gluconeogenesis will be constitutively repressed and a child with PEPCK deficiency should remain well. However, in the stressed state, together with reduced oral intake for a prolonged period of time it is conceivable that the presence of PEPCK deficiency would impair the body's ability to cope with prolonged starvation as gluconeogenesis from oxaloacetate cannot be properly switched on. This may explain why this child presented only after a prolonged gastroenteritic illness and why the clinical condition, as well as metabolic abnormalities, improved rapidly upon the provision of intravenous dextrose. Additionally, some gluconeogenesis may occur from glycerol independent of PEPCK activity. This patient did not show glyceroluria, suggesting this pathway should not be defective. It is, however, possible that insufficient glycerol was being delivered for glucose production because PEPCK-C should also help to produce glycerol (glyceroneogenesis) [31].

Oxaloacetate is also a component of the TCA cycle and therefore PEPCK also has an important function as a cataplerotic reaction for the TCA cycle [32]. Consequently PEPCK deficiency would be expected to lead to an accumulation of oxaloacetate and pyruvate within the cell. Oxaloacetate accumulation within the mitochondrion could conceivably lead to secondary inhibition of the TCA cycle and mitochondrial respiratory chain – and may underlie the liver failure and markedly raised TCA metabolites in the child's urine. There is evidence to support these secondary effects of PEPCK deficiency from a zebrafish *PCK1*-knockout model, which demonstrates significant malate accumulation [33]. However why this should happen in the presence of intact *PCK2* (and thus normal mitochondrial PEPCK activity) is not clear – though the in vivo role for mitochondrial PEPCK has yet to be fully elucidated and a number of roles for this isoform outside of glucose metabolism have been proposed [34].

As possible explanation for the amino acid profile reminiscent of a proximal UCD, we propose that accumulation of high concentrations of other TCA cycle metabolites such as alpha-ketoglutarate could conceivably trigger increased flux through alternative cataplerotic reactions

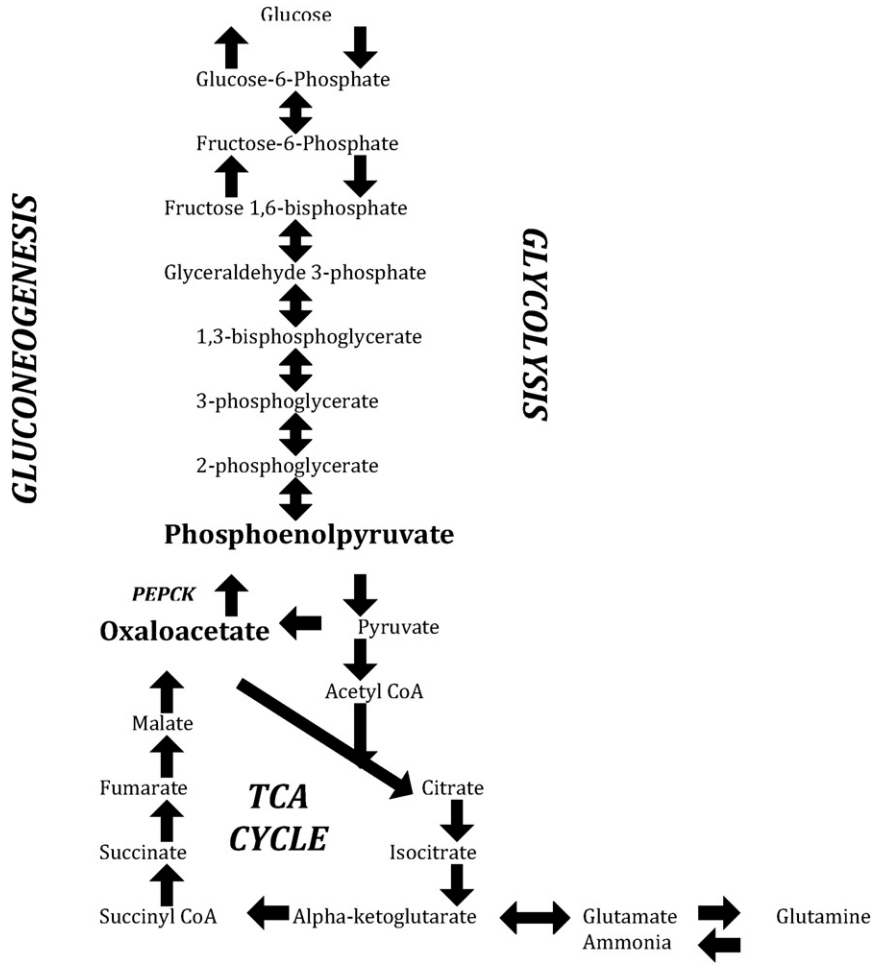

Fig. 4. The role of PEPCK in gluconeogenesis and TCA cycle cataplerosis.

such as the reversible glutamate dehydrogenase and glutamine synthase which would metabolise alpha-ketoglutarate to glutamate and glutamine in the presence of moderate hyperammonaemia [32]. In keeping with this hypothesis, markedly raised plasma glutamine in the face of only modest hyperammonaemia was seen in this child during illness. Unlike typical UCDs, the amino acid profile remained normal upon this child's return to a non-stressed metabolic state.

The genetic and biochemical results on this child suggest a causative deficiency of the PEPCK for the liver failure seen in this child. The metabolic abnormalities observed in this child rapidly normalized on the provision of dextrose, which is consistent with the hypothesised effect of PEPCK deficiency. This presentation may be under-recognised in children presenting with liver failure in whom organic acids and amino acids collected during the acute episode are abnormal, but normalize following recovery.

Prevention of gluconeogenesis by the avoidance of fasting and use of a glucose polymer emergency regimen appears to have prevented further decompensations in this child.

In conclusion, PEPCK deficiency may be an under-recognised but treatable cause of childhood liver failure associated with illnesses causing prolonged starvation. We propose that PEPCK deficiency should be considered in the young child with unexplained liver failure which rapidly responds to supportive therapy and especially where there are marked, transient accumulations of TCA cycle metabolites on urine organic acid analysis and/or an amino acid profile with hyperammonaemia suggestive of a proximal UCD. If suspected, intravenous administration of dextrose should be initiated. Long-term management comprising avoidance of fasting with the provision of a glucose polymer emergency regimen for illness management may be sufficient to prevent future episodes of liver failure. Our case report provides further insights into the (patho-)physiology of energy metabolism, confirming the power of WES analysis of unexplained biochemical phenotypes.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2016.03.001.

Funding

This work was supported by funding from the BC Children's Hospital Foundation (1st Collaborative Area of Innovation, www.tidebc.org), Genome BC (SOF-195 grant), and the Canadian Institutes of Health Research (#301221 grant). Informatics infrastructure was supported by Genome BC and Genome Canada (ABC4DE Project). CvK is a Michael Smith Foundation for Health Research Scholar. CJDR is supported by a CIHR New Investigator award.

Acknowledgments

We gratefully acknowledge the family for their participation in this study; Mr. B. Sayson and Ms. A. Ghani for consenting and coordination of sample collection; Mrs. X. Han for Sanger sequencing; Mrs. M. Higginson for gDNA extraction, sample handling and technical data (University of British Columbia, Vancouver, CA).

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