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Cytosolic phosphoenolpyruvate carboxykinase deficiency presenting with acute liver failure following gastroenteritis

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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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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.