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Novel homozygous *PCK1* mutation causing cytosolic phosphoenolpyruvate carboxykinase deficiency presenting as childhood hypoglycemia, an abnormal pattern of urine metabolites and liver dysfunction

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ABSTRACT

Clinical and laboratory data were collected from three Finnish patients including a sibling pair and another unrelated child with unexplained childhood hypoglycemia. Transient elevation of alanine transaminase, lactate and tricarboxylic acid cycle intermediates, especially fumarate, were noticed in urine organic acid analysis. Exome sequencing was performed for the patients and their parents. A novel homozygous *PCK1* c.925G>A (p.G309R) mutation was detected in all affected individuals. COS-1 cells transfected with mutant *PCK1* transcripts were used to study the pathogenic nature of the detected variant. The COS-1 transfected cells showed the mutant gene to be incapable of producing a normally functioning cytosolic phosphoenolpyruvate carboxykinase (PEPCK) enzyme. This report further delineates the clinical phenotype of isolated cytosolic PEPCK deficiency and offers a metabolic pattern helping to recognize these patients. Cytosolic PEPCK deficiency should be considered in the differential diagnosis of children presenting with hypoglycemia, hepatic dysfunction and elevated tricarboxylic acid intermediates in urinary organic acid analysis.

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1. Introduction

Gluconeogenesis is an enzymatic process responsible for ensuring the availability of glucose for the needs of the body during prolonged periods of inadequate carbohydrate intake. The liver is mainly responsible for gluconeogenesis, for example by catabolizing glucogenic amino acids. Phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) catalyzes part of the cataplerotic process where the 4- and 5-carbon intermediates formed in the catabolism of amino acids are removed from the tricarboxylic acid (TCA) cycle. PEPCK has two isoforms, cytosolic PEPCK–C (MIM 261680) and mitochondrial PEPCK-M (MIM 261650).

Mitochondrial PEPCK deficiency has been described in a few cases, but none have been defined at the molecular level. In all cases, diagnosis was made by deficient PEPCK activity in fibroblasts, which has 100% PEPCK-M isoform [1–4]. The general clinical presentations were hypotonia, hepatomegaly, failure to thrive, lactic acidosis and hypoglycemia. Deficiency of the cytosolic form of the PEPCK is an even rarer inherited metabolic disorder of impaired gluconeogenesis caused by mutations in *PCK1* (phosphoenolpyruvate carboxykinase, MIM 614168). Previous publications on biochemically confirmed cytosolic PEPCK deficiency

Abbreviations: ACMG, American College of Medical Genetics and Genomics; ALT, Alanine aminotransferase; CADD, Combined Annotation Dependent Depletion; MAF, Minor allele frequency; OAA, Oxaloacetate; PC, Pyruvate carboxylase; PEP, Phosphoenolpyruvate; PEPCK, Phosphoenolpyruvate carboxykinase; RI, Reference interval; TCA, Tricarboxylic acid; WES, Exome sequencing.

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have described a severe clinical picture with persistent neonatal hypoglycemia, lactic acidosis and liver failure, leading to neurological degeneration [5–8]. Only recently, there were two reports published on genetically confirmed deficiency (i.e. due to recessive *PCK1* mutations). The first was a sibling pair who had the cytosolic PEPCK deficiency either in conjunction with Smith-Magenis syndrome or *N*-methyl-D-aspartate (NMDA) receptor glutamate insensitivity [9]. The second was an isolated PEPCK deficiency due to homozygous *PCK1* deletion presenting with transient severe liver failure (including a biochemical profile of hyperammonemia, lactic acidosis and elevated TCA metabolites) elicited by gastroenteritis responsive to a dextrose infusion [10]. Infections and fasting have been suggested to be driving forces for symptomatic periods.

Here we further expand the phenotype by reporting the data of three previously healthy individuals (2 siblings and 1 unrelated singleton) with a novel homozygous *PCK1* mutation who presented with hypoglycemia in early childhood, mostly without an obvious predisposing or eliciting factor.

2. Methods

2.1. Study subjects

The patients were seen at the Clinic for Children and Adolescents and the Department of Clinical Genetics of Oulu University Hospital in 2013–2016 (patients 1.1), 2015–2016 (patient 1.2) and 2014–2016 (patient 2). Their examinations included clinical and laboratory assessments, biochemical and molecular genetic analyses. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Prior to the study, the parents gave written informed consent for their children to participate in the study approved by the Ethics Committee of the Northern Ostrobothnia Hospital District (permit no: 51/2008, and the amendments 2011 and 2014).

2.2. DNA extraction

DNA was extracted from the peripheral blood of the three affected patients, their healthy parents and the two healthy unaffected siblings using standard methods, based on the QIAsymphony DSP DNA Mini Kit (Qiagen, Valencia, CA) on the QIAsymphony instrument. Prior to the analysis the DNA quality and concentration was determined photometrically (OD260/OD280 1.8–2.0).

2.3. Exome sequencing (WES) and Sanger analysis

WES was performed commercially in Centogene AG (Rostock, Germany), in concordance to the provisions of the German Gene Diagnostic Act (Gendiagnostikgesetz) and the General Data Protection Act (Bundesdatenschutzgesetz) to guarantee the confidentiality and protection of data. Sequencing was performed using Illumina's Nextera Rapid Capture Exome Kit and HiSeq4000 sequencers (Illumina, Inc., San Diego, CA). Data was processed using bcl2fastq software 2.17.1.14 (Illumina, Inc., San Diego, CA), and fed to an in-house pipeline based on the 1000 Genomes Project data and Genome Analysis Toolkit (GATK) best practice recommendations. Variants were called using Burrows-Wheeler Alignment (BWA) software with the Maximal Exact Matches (MEM) algorithm and GATK HyplotypeCaller, freebayes and samtools. Variants were annotated using Annovar Alamut version 2.4.5 (Interactive Biosoftware, Rouen, France) and in-house ad hoc bioinformatics tools and compared to the in-house mutation database (CentoMD®), The Human Gene Mutation Database (HGMD®), and ClinVar[16]. Variants were filtered to keep only those with ≥10× depth of coverage, ≥20% of total reads and <1% in the Exome Aggregation Consortium database (ExAC, http://exac.broadinstitute.org), and CentoMD® (http://www.centomd.com). Prioritized variants were evaluated based on mode of inheritance and compatibility with the clinical phenotype provided for the index. All clinical features provided were used for each individual case, and, in addition, the HPO ontology was implemented to classify the patient phenotypes. Selected candidate pathogenic, likely pathogenic, and VUS variants were confirmed by conventional PCR amplification and Sanger sequencing. Sanger sequencing of the identified *PCK1* mutation of further family members was performed to confirm the segregation of the variant with the disease phenotype.

2.4. PCK1 G309R plasmid construction, transfection and PEPCK enzyme activity measurements

Human cytosolic phosphoenolpyruvate carboxykinase 1 (*PCK1*) full-length cDNA (GenBank accession # NM_002591) was cloned in pcDNA3.1 mammalian expression vector (GenScript). To create the G309R mutant *PCK1*, a nucleotide fragment containing *PCK1* c.925A (p.309R) was synthesized (GenScript), and then used to replace the wild type *PCK1* sequence by subcloning. Another mutant *PCK1* with a 12 bp deletion (c.1319_1330del GTGTCCCTCTAG; p.440_444del GVPL) was created as described previously [10], and used as a negative control in the present study. All cloned mutants were confirmed by Sanger sequencing. COS-1 cells were transfected with pcDNA3.1 plasmids using lipofectamine 2000 reagent (Life Technologies) for 48 h. The whole cell lysates were used for Western Blot analysis and for PEPCK enzyme activity measurements. PEPCK enzyme measurements were performed in cells as previously described [10].

2.5. Western blot

Western blot analysis of PEPCK protein was performed as described previously [10]. Briefly, ~20 μg of protein from COS-1 cell lysates was fractionated by electrophoresis through a 10% SDS polyacrylamide gel, transferred to PVDF membrane, and then immunoblotted with polyclonal anti-PCK1 antibody (Proteintech; 16754-1-AP) at a 1:1000 dilution. GAPDH was immunoblotted with anti-GAPDH antibody (Millipore, MAB374; 1:5000 dilution) on the same membranes as a loading control.

3. Results

3.1. Case reports

Patient 1.1 (Fig. 1, family 1, affected individual II-3), is the third child of distantly consanguineous parents of Finnish origin, born at term after an uncomplicated pregnancy with Apgar scores of 9/10. She needed a glucose infusion during the first 24 h of life due to low blood glucose levels of 0.9 mmol/l (reference interval RI 4.2–6.3 mmol/l), tested because of tremor and irritability. Her growth and psychomotor development were normal. At the age of 14 months she was noticed to be unusually drowsy and poorly responsive when waking up in the morning after an overnight fast of about 12 h. The drowsiness did not resolve with more sleep, so an ambulance was called. She was able to drink a bit of milk before the arrival of the ambulance, after which the glucose was measured at 1.8 mmol/l. She had no signs of infection, and infection parameters remained low. At admission, base excess was −11 and pH 7.33. Blood lactate and alanine aminotransferase (ALT) were mildly elevated (Table 1). Plasma ammonia was 25 μmol/l (normal < 50 μmol/l). In plasma quantitative amino acid analysis glutamine was slightly elevated while citrulline and arginine were within normal ranges (Table 1). Semiquantitative analysis of urine organic acids by gas chromatography/mass spectrometry was performed two weeks later, after a 20 h fasting test during which blood glucose was 3.2 mmol/l. Urine organic acids demonstrated prominent TCA cycle metabolites (Table 1): especially fumarate but also succinate and 2-ketoglutarate. 3-hydroxybutyrate excretion was intermediately increased while several dicarboxylic acids,

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