


# Maternal Primary Carnitine Deficiency and a Novel Solute Carrier Family 22 Member 5 (SLC22A5) Mutation

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## Abstract

Primary carnitine deficiency (PCD) is a rare autosomal recessive disorder caused by loss of function mutations in the solute carrier family 22 member 5 (SLC22A5) gene that encodes a high-affinity sodium-ion–dependent organic cation transporter protein (OCTN2). Reduced carnitine transport results in diminished fatty acid oxidation in heart and skeletal muscle and carnitine wasting in urine. We present a case of PCD diagnosed in an adult female after a positive newborn screen (NBS) for PCD that was not confirmed on follow-up testing. The mother was referred for evaluation of persistent fatigue and possible hypothyroidism even though all measurements of thyroid-stimulating hormone were well within the range of 0.4 to 2.5 mIU/L expected for reproductive-age women. She was found to have unequivocally low levels of both total carnitine and carnitine esters, and genetic testing revealed compound heterozygosity for 2 SLC22A5 mutations. One mutation (c.34G>A [p.Gly12Ser]) is a known missense mutation with partial OCTN2 activity, but the other mutation (c.41G>A [p.Trp14Ter]) is previously unreported and results in a premature stop codon and truncated OCTN2. This case illustrates that some maternal inborn errors of metabolism can be identified by NBS and that maternal carnitine levels should be checked after a positive NBS test for PCD.

## Keywords

maternal primary carnitine deficiency, SLC22A5 mutation

## Introduction

Carnitine ( $\beta$ -hydroxy- $\gamma$ -trimethylammonium butyrate) is a hydrophilic quaternary amine that plays an essential role in the transfer of long-chain fatty acids to mitochondria for  $\beta$ -oxidation. Primary carnitine deficiency (PCD) is an autosomal recessive disorder caused by loss of function mutations in the solute carrier family 22 member 5 (SLC22A5) gene on chromosome 5q31 that codes for organic cation/carnitine transporter 2 (OCTN2).<sup>1,2</sup> OCTN2 is expressed in high amounts in skeletal muscle, myocardium, and kidneys, and defective OCTN2 carnitine transport activity results in urinary carnitine wasting, low circulating carnitine and acyl-carnitine levels, decreased intracellular accumulation of carnitine, and impaired long-chain fatty acid oxidation.<sup>3</sup>

The incidence of PCD in the United States is approximately 1:140 000, and PCD is typically diagnosed in early childhood through newborn screening (NBS) or presentation in the first 2 years of life with an episode of hypoketotic hypoglycemia, hyperammonemia, abnormal liver function tests, and encephalopathy provoked by acute illness that leads to poor eating.<sup>4,5</sup> Children with PCD are also

susceptible to cardiomyopathy that is poorly responsive to standard interventions and progresses to refractory heart failure requiring cardiac transplant or resulting in death if not treated with carnitine supplements.<sup>6</sup> Both children and adults with PCD may develop long QT syndrome and ventricular tachyarrhythmias.<sup>7</sup> Other reported manifestations of PCD in children include anemia, proximal muscle weakness, and developmental delays.<sup>8</sup>

Postpartum women are occasionally diagnosed with PCD when a positive NBS cannot be confirmed on follow-up testing.<sup>9–11</sup> Most adult patients are asymptomatic at time of diagnosis, though some report improved exertional tolerance after treatment with carnitine supplements.<sup>11</sup> All adults with

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newly diagnosed PCD should receive carnitine supplements due to increased risk of cardiac arrhythmia and sudden death.<sup>9-11</sup> We present a case of PCD diagnosed in an adult female after positive NBS. The patient was discovered to be a compound heterozygote for 2 SLC22A5 mutations, including one that appears to be a novel mutation.

## Case Presentation

A 26-year-old female was referred for evaluation of persistent fatigue, unintentional weight gain, and possible hypothyroidism. A right thyroid lobectomy had been performed 6 years prior to referral for management of a uninodular goiter, and the nodule was confirmed to be benign on postoperative histopathology. All thyroid-stimulating hormone measurements following surgery were in the range of 0.4 to 2.5 mIU/L (0.45-5.33). Fatigue mainly manifested as poor exertional tolerance and started in early adolescence, well before thyroid surgery. The patient reported 7 years and nearly 70 pounds of unintentional weight gain, and she denied any sustained or current dietary restrictions such as veganism. Computed tomography of the abdomen ordered to evaluate an episode of abdominal pain and diarrhea shortly before the patient's office visit showed mild hepatic steatosis. Body mass index was 38 kg/m<sup>2</sup>, a well healed low neck incision was observed, and the right thyroid lobe and isthmus were absent to palpation. The remainder of history and physical examination was unremarkable.

The patient and her husband had 2 children. The first child tested positively on NBS for PCD, but a repeat measurement of serum carnitine level after 2 weeks of exclusive bottle feeding was within the laboratory reference range. The second child also had a positive NBS for PCD, and serum carnitine level remained low while he was breast fed. This history prompted measurements of the patient's carnitine and carnitine ester levels, and a pattern consistent with PCD was discovered (Tables 1 and 2). Genetic testing was subsequently arranged, and the patient was found to have 2 SLC22A5 mutations by Sanger DNA sequencing (Table 3). The first mutation has been reported in cases of lethal infant-onset PCD, though in vitro assays show this mutation reduces OCTN2 activity only by approximately half.<sup>12-15</sup> The second mutation results in a premature stop codon that results in a highly truncated OCTN2 mRNA that undergoes nonsense-mediated decay and is previously unreported in the ARUP SLC22A5 database, gene-specific databases (Global Variome shared Leiden Open Variation Database), general population databases (Exome Variant Server and Genome Aggregation Database), or peer-reviewed literature.<sup>16-19</sup>

The patient's symptoms, coupled with low total and acyl carnitine levels, were felt to indicate she is a compound heterozygote for her SLC22A5 mutations. However, since the phase (cis or trans) of genetic variants cannot be established by Sanger sequencing, buccal swabs were obtained from the

**Table 1.** Carnitine Levels.

| Parameter                         | Results (μM) | Reference range |
|-----------------------------------|--------------|-----------------|
| Evaluation                        |              |                 |
| Total carnitine (#1)              | 13           | 25-58           |
| Total carnitine (#2)              | 13           |                 |
| Carnitine esters                  | 2            | 4-13            |
| Treatment                         |              |                 |
| Total carnitine (#1) <sup>a</sup> | 21           | 25-58           |
| Total carnitine (#2) <sup>b</sup> | 40           |                 |
| Carnitine esters <sup>a</sup>     | 5            | 4-13            |

<sup>a</sup> Measured during treatment with carnitine at 20 mg/kg/d in 4 divided doses.

<sup>b</sup> Carnitine advanced to 50 mg/kg/d.

**Table 2.** Effects of Carnitine Pathway Defects on Circulating Carnitine Levels.

| Defect | Total/free carnitine | Carnitine esters |
|--------|----------------------|------------------|
| OCTN2  | ↓                    | ↓                |
| CPT I  | ↑                    | ↓                |
| CACT   | ↓                    | ↑                |
| CPT II | ↓                    | ↑                |

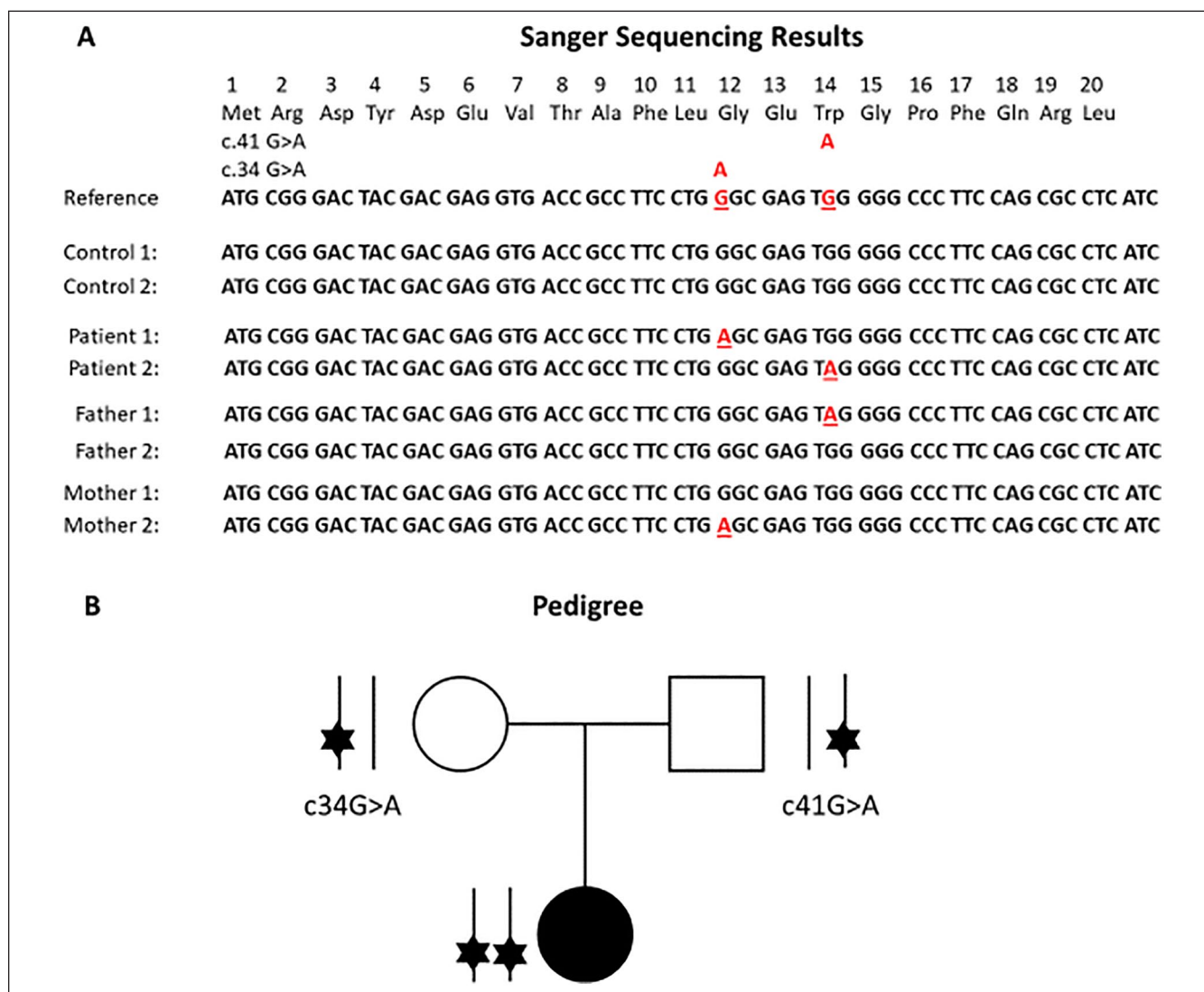
Abbreviations: OCTN2, cation transporter protein; CPT, carnitine palmitoyltransferase II; CACT, carnitine-acylcarnitine translocase.

patient and her biological parents for DNA sequencing in the laboratory of one of the authors (AW). Genomic DNA was isolated using the Isohelix Buccal-Prep Plus DNA Kit (Boca Scientific Inc) according to the manufacturer's instructions. Sequences for exon 1 of the SLC22A5 gene were polymerase chain reaction (PCR) amplified from high-molecular-weight genomic DNA (25 ng) using forward primer (5'-GCT CTG TGG GCC TCT GAG-3'), reverse primer (5'-CAC CTC GGT CAC AAT GGT G-3'), and MyFi Mix (Meridian Bioscience). PCR conditions were 98 °C-30 seconds followed by 35 cycles at 98 °C-5 seconds, 63 °C-15 seconds, 72 °C-15 seconds, and final extension at 72 °C-5 minutes before terminating at 4 °C. A 420-bp amplicon was extracted from agarose gel using the GeneJET Gel Extraction Kit and inserted into the pCR2.1 TOPO/TA cloning vector (both from Thermo Fisher Scientific). Plasmids isolated from bacterial colonies were sent to Genewiz for Sanger sequencing. Trace files were analyzed using Geneious software package version 6.1.2 (Biomatters Ltd) and compared with SLC22A5 PCR products generated from genomic DNA of human CD34<sup>+</sup> cells, which served as a control, and reference sequence HGNC: 10969. The patient was confirmed to be a true compound heterozygote for her SLC22A5 mutations reported by commercial gene sequencing. Specifically, the patient's mother was the source of the c.34G>A (p.Gly12Ser) mutation, and her father was the source of the c.41G>A (p.Trp14Ter) mutation. Sanger sequencing results and the patient's pedigree are presented in Figure 1.

**Table 3.** Patient's SLC22A5 Mutations.

| Mutation             | Effect on OCTN2   | Clinical effects                               |
|----------------------|---|--|
| c.34G>A (p.Gly12Ser) | Activity reduced by ~50%  | Reported in some lethal infant-onset PCD cases |
| c.41G>A (p.Trp14Ter) | Premature stop codon; truncated protein with no activity or mRNA subject to nonsense-mediated decay | First report of this mutation                  |

Abbreviations: SLC22A5, solute carrier family 22 member 5; OCTN2, cation transporter protein; PCD, primary carnitine deficiency.



**Figure 1.** DNA sequencing of exon I from the SLC22A5 gene (A) demonstrating the patient to be a compound heterozygote for mutations c.41G>A and c.34G>A. The patient's father is heterozygous for the c.41G>A nonsense mutation, and her mother is heterozygous for the c.34G>A missense mutation. The c.41G>A variant results in a premature stop codon in place of Trp at amino acid 14, and the C.34G>A variant results in substitution of Ser for Gly at amino acid 12. The pattern of inheritance is more clearly presented in the patient's pedigree (B).

No conduction abnormalities were observed on an electrocardiogram, and an echocardiogram showed an anatomically normal heart with preserved left ventricular ejection fraction (60% to 65% by visual estimation). Carnitine

supplementation was started at 20 mg/kg/d in 4 divided doses and adjusted to 50 mg/kg/d to achieve serum total and acyl carnitine levels within their respective references ranges (Table 1). The patient reported significant improvements

in sense of well-being and exertional tolerance after starting treatment with carnitine supplements. She saw a dietitian, began a program of regular aerobic exercise, started treatment with the Saxenda preparation of liraglutide, and achieved an intentional 15 pound weight loss at a 4 month follow-up appointment.

## Discussion

Carnitine was discovered in vertebrate muscle in 1905, and it was found to play a role in fat metabolism with the discovery that yellow meal worms (*Tenebrio molitor*) could not utilize fat stores when starved without carnitine.<sup>20</sup> Carnitine was subsequently demonstrated to stimulate fatty acid oxidation in liver homogenates by facilitating transport of activated long-chain fatty acid acyl-CoA thioesters into mitochondria.<sup>21</sup> Carnitine is a conditionally essential nutrient; consumption of meat and dairy products provides approximately 75% of daily requirements in a typical diet, though carnitine can also be synthesized from the amino acids lysine and methionine.<sup>3,22</sup>

PCD is a recently recognized metabolic disorder, with the first case published in 1973.<sup>23</sup> A clear link between PCD and impaired carnitine transport was established 15 years later, and defects of OCTN2 activity were confirmed to be causal for PCD in 1999.<sup>1,24</sup> The gene SLC22A5 (MIM# 603377) encodes for OCTN2 and is located on chromosome 5q31. The transporter is composed of 557 amino acids and 12 transmembrane domains, with the amino- and carboxy-termini both facing the cytoplasm analogous with other organic cation transporters.<sup>25</sup> The amino-terminus and transmembrane domains 7, 9, and 10 are essential for carnitine recognition, and the carboxy-terminus is required for transmembrane transfer of carnitine.<sup>26</sup> Nonsense and frameshift mutations in SLC22A5 typically result in lower carnitine transport and are more prevalent in symptomatic patients, while missense mutations and in-frame deletions are associated with better preservation of carnitine transport and are more commonly found in minimally symptomatic individuals or asymptomatic mothers of unaffected infants identified by NBS.<sup>27</sup>

Carnitine is transported from placenta to fetus during intrauterine life, and low carnitine levels measured on NBS reflect the mother's deficiency in cases of maternal PCD.<sup>28,29</sup> In 2 recent, large NBS series from Zhejiang Province (over 3.4 million newborn screens) and Xuzhou area (over 230 000 newborn screens) in China, the incidences of maternal PCD were 1.8/100 000 and 2.5/100 000, respectively.<sup>30,31</sup> A total of 69 maternal PCD cases were diagnosed between the 2 series; in Zhejiang Province, over 90% of maternal mutant SLC22A5 alleles were missense mutations, and all cases in Xuzhou area were due to missense mutations. The most common variant was c.1400C>G (p.Ser467Cys). All cases of maternal PCD in a series of 5 patients in the United States were

due to missense mutations, with c.1195C>T (p.Arg399Trp) and c.248G>T (p.Arg83Leu) the most common SLC22A5 variants.<sup>9</sup>

Our patient's case is unusual in that, based on gene sequencing, laboratory, and clinical findings, she is a compound heterozygote for a previously unreported nonsense mutation and a missense mutation of indeterminate significance (Figure 1). The c.41G>A (p.Trp14Ter) mutation results in a premature stop codon that is predicted to result in either a highly truncated OCTN2 of only 13 amino acids with no carnitine transport activity or no expression of protein due to nonsense-mediated mRNA decay. Other nonsense mutation variants downstream from this patient's mutation have been documented to occur in cases of PCD with typical clinical manifestations.<sup>13,15,32</sup> Function of the patient's missense mutation variant (c.34G>A [p.Gly12Ser]) has been studied in Chinese Hamster Ovary cells stably transfected with p.Gly12Ser in an OCTN2-EGFP expression vector, and carnitine transport was 51.7% of wild-type OCTN2.<sup>15</sup> However, the c.34G>A mutation is identified as damaging by both the Polyphen-2 and SIFT programs for predicting the pathogenicity of missense variants, and this missense SLC22A5 mutation has been found in cases of infant lethal PCD.<sup>12,14,15</sup>

Based on what is published regarding nonsense SLC22A5 mutations and the apparent function of the patient's missense OCTN2 variant in tissue culture studies, it is anticipated that the patient would have approximately 25% of expected carnitine transport capacity. This likely explains low total carnitine and carnitine ester levels and the relatively mild phenotype that escaped diagnosis until her children screened positively for PCD. Fortunately, the patient had no measureable cardiac manifestations of PCD, and it is unclear if mild nonalcoholic fatty liver was due specifically to PCD or obesity. The patient's improved well-being and exertional tolerance after starting carnitine supplementation has been documented in other cases of adult PCD, though her carnitine dose (50 mg/kg/d) is below what is typically required to achieve normal carnitine levels (100-200 mg/kg/d).<sup>11</sup>

PCD is an autosomal recessive disorder of carnitine transport due to mutations in SLC22A5 that is detected on NBS or manifests clinically in early childhood. Maternal cases of PCD are usually diagnosed when apparent neonatal PCD on NBS cannot be confirmed, and most cases are due to missense mutations. Our case is an important contribution to the published literature on maternal PCD for the following reasons: (1) it appears to be the first report of the patient's nonsense mutation (c.41G>A), expanding the spectrum of known SLC22A5 variants; (2) the patient's survival to adulthood with the c.34G>A SLC22A5 variant as her only functional allele confirms that this mutation is not invariably infant lethal; and (3) the metabolic and clinical phenotype of this first occurrence of compound heterozygosity for

the patient's missense and nonsense SLC22A5 mutations has been clearly documented, with the patient's degree of impaired carnitine transport apparently enough to cause reduced exertional tolerance.

Though maternal PCD is rare, recognition is important for interpreting the significance of NBS results, potentially determining the cause of nonspecific symptoms like fatigue as in this patient's case, and identifying women who are candidates for carnitine supplementation. Gene sequencing in these cases helps define the prevalence of known SLC22A5 mutations and identify novel variants that may be disease-causing.

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The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Ethics Approval

The Southern Illinois School of Medicine does not require ethical approval for reporting individual cases or case series.

### Informed Consent

Verbal informed consent was obtained from the patient and her parents for their anonymized information to be published in this article.

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