Severe Hyperammonemic Encephalopathy Requiring Dialysis Aggravated by Prolonged Fasting and Intermittent High Fat Load in a Ramadan Fasting Month in a Patient with *CPTII* Homozygous Mutation

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Abstract Background: Carnitine palmitoyltransferase II (CPTII) deficiency is a mitochondrial fatty acid oxidation disorder that can present antenatally as congenital brain malformations, or postnatally with lethal neonatal, severe infantile, or the most common adult myopathic forms. No case of severe hyperammonemia without liver dysfunction has been reported.

Case Presentation: We described a 23-year-old man who presented to the emergency department with seizures and was found to have markedly elevation of serum ammonia. Continuous renal replacement therapy was initiated with successfully decreased ammonia to a safety level. He had a prolonged history of epilepsies and encephalopathic attacks that was associated with high ammonia level. Molecular diagnosis revealed a homozygous mutation in *CPTII*. The plasma acylcarnitine profile was consistent with the diagnosis. Failure to produce acetyl-CoA, the precursor of urea cycle from fatty acid in prolonged fasting state in Ramadan month, worsening mitochondrial functions from

circulating long chain fatty acid and valproate toxicities were believed to contribute to this critical metabolic decompensation.

Conclusion: Fatty acid oxidation disorders should be considered in the differential diagnosis of hyperammonemia even without liver dysfunction. To our knowledge, this is the first case of CPTII deficiency presented with severe hyperammonemic encephalopathy required dialysis after prolonged religious related fasting.

Background

Carnitine palmitoyltransferase II (CPTII) deficiency (MIM: 600649) is a disorder of mitochondrial beta-oxidation of long chain fatty acid (Bonnefont et al. 2004). The affected patients can be detected antenatally with brain malformations (Boemer et al. 2016) or postnatally ranged from lethal neonatal disease (Sigauke et al. 2003), severe infantile hepatocardiomyopathy form (Bouchireb et al. 2010), and adult myopathic form (Joshi et al. 2014). The adult form is the most common type in which patients present with recurrent rhabdomyolysis. However, patients can present with the symptoms from 2 years of age but mostly manifest in teenagers or adults. Most of the patients with neonatal form died early in their life except a case surviving its second birthday (Ikeda et al. 2017). Severe infantile form of CPTII deficiency is one of the causes of sudden unexpected deaths of childhood period. Patients can be suffered from recurrent attacks of seizures, or hypoglycemia if prolonged fasting, or stress that induces catabolism. Many reports show that these patients survived to teenage years with specific treatment with medium chain triglycerides supplement and restriction of total fat intake. Hyperammonemia

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can be the result of liver injury during the illness or as a result of lack of acetyl Co-A, the precursor of N-acetylglutamate, the intermediate metabolite in urea cycle (Walser and Stewart 1981). Severe hyperammonemic encephalopathy required acute dialysis has never been reported in patient with CPT II deficiency. Here, we reported a patient with atypical feature of CPTII deficiency with non-cirrhotic hyperammonemia mimicking urea cycle disorder after intermittent fasting during Ramadan month and intermittent loading of high fat diet. The molecular diagnosis solved the puzzle and led to direct therapy according to the pathophysiology of the disease diagnosed.

Subject

We studied a Thai patient who attended the Genetics Clinic as the part of undiagnosed disease program at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand. The medical data, pedigree, physical examinations, and laboratory results were recorded. The written informed consent and parental consent (for the proband) were obtained after explanation of the possible consequences of this study.

Genomic DNA Preparation and Whole-Exome Sequencing

To perform genetic analysis, genomic DNA was isolated from peripheral blood leukocytes using a Puregene Blood kit (Qiagen, Hilden, Germany). The genomic DNA was sent to Macrogen, Inc. (Seoul, South Korea) for whole-exome sequencing (WES). DNA was captured using a SureSelect Human All Exon version 4 kit (Agilent Technologies, Santa Clara, CA) and sequenced on a HiSeq2000 instrument. Base calling was performed and quality scores were analyzed using Real Time Analysis software version 1.7. Sequence reads were aligned against the University of California Santa Cruz human genome assembly hg19 using Burrows-Wheeler Alignment software (bio-bwa.sourceforge.net/). Single-nucleotide variants (SNVs) and insertions/deletions (Indels) were detected by SAMTOOLS (samtools.sourceforge.net/) and annotated against dbSNP & the 1000 Genomes Project. After quality filtering, we looked for variants located in the coding regions of known genes causing urea cycle disorders, fatty acid oxidation disorders, aminoacidurias, and organic acidemias for all potential pathogenic SNVs and Indels. Variant calling exclusion criteria were (a) coverage <10×; (b) quality score <20; (c) minor allele frequency <1% in the 1000 Genomes Project; and (d) noncoding variants and synonymous exonic variants. The remaining variants were subsequently filtered

out if they were present in our in-house database of 719 unrelated Thai exomes. Existing SNVs or known pathogenic mutations were filtered out of the Exome Aggregation Consortium database (exac.broadinstitute.org).

Case History

A 23-year-old Thai Muslim male was referred to King Chulalongkorn Memorial Hospital Emergency Room after the episodes of generalized tonic-clonic seizures. He received diazepam, valproic acid, and phenytoin intravenously before transferring to our hospital. Capillary glucose was measured 67 mg/dL and 10% dextrose solution infusion was initiated. Patient gained his consciousness after the seizure episode. At the emergency department, he started to develop another episode of general tonic-clonic seizures. Intravenous levetiracetam was given. Patient was intubated endotracheally and was admitted to the intensive care unit. Serum ammonia was elevated to more than 800 $\mu\text{mol/L}$ (ten times above the upper normal limit). A nephrologist was consulted for prompt continuous hemofiltration. After 8 h, serum ammonia was reduced to 110 $\mu\text{mol/L}$ when the hemofiltration was discontinued. The patient's ammonia was normalized within 24 h after the first measurement. He gained consciousness, was extubated, and as back to his baseline without neurological sequelae. However, patients developed rhabdomyolysis with CPK of more than 84,000 units/mL. Retrospectively, his father reported that the patient practiced fasting during the Ramadan fasting month and celebrated out of the fasting periods with a big feast of food with high fat contents (skin-on chicken with oil-cooked rice). A few days prior to this episode, he complained of generalized muscle pain especially at paraspinal muscles.

Patient's history review revealed that he was a fraternal twin born to consanguineous parents (Fig. 1). His fraternal twin sister is healthy. The family denied other members with the same medical issues besides an older sister of the patient who died at 1 year of age from uncontrolled seizure. The patient started developing seizure at 1 year of age. There were several occasions which he had elevations of blood ammonia [216 $\mu\text{g/dL}$ (30–85), 206 and 430 $\mu\text{mol/L}$ (9–33)]. The plasma amino acid was nonspecific with generalized low levels of all plasma amino acids including citrulline, ornithine, and argininosuccinate. Urine organic acid analysis did not show any increased excretion of specific metabolites. Liver enzymes were normal. He previously had controllable seizures. He experienced many episodes of recurrent alteration of consciousness related to large amounts of animal or plant protein intake (chicken, fish, and nuts) but he tolerated well with his favorite food: quail eggs. He had mild intellectual disability with general

Fig. 1 Family pedigree of the proband with consanguineous parents and a deceased sibling suspected to be affected with the same disease

IQ of 60 and developed aggressive behavior and video games addiction. He was controlled with rivotril and valproate.

Fortunately, the molecular diagnosis was available for this patient at the time of patient's arrival at the emergency department. A homozygous missense mutation (chromosome 1:53679221) c.1931T>C, p.L644S of *CPTII* was identified by whole exome sequencing with the total read depths of 51. This variant has not been reported in the ExAC database, 1000 genomes or Thai in-house exome database and was predicted to be deleterious/damaging by multiple prediction programs including SIFT, PolyPhen, and M-CAP. This variant is previously reported in a sudden unexpectedly dead infant as a compound heterozygous with p.F388Y (Yamamoto et al. 2011). Our patient was started on formula diet with low fat content supplemented with medium chain triglycerides and transitioned to natural food prior to discharge from the hospital under the guidance of dietician.

Discussion

We described a patient with *CPTII* deficiency who presented with severe hyperammonemia and seizures responded with extracorporeal elimination by hemofiltration. This is an atypical presentation of *CPTII* deficiency, a fatty acid oxidation disorder. This high level of ammonia is far beyond the transient hyperammonemia post seizure which does not require any treatment (Hung et al. 2011). The mechanism of this patient's massive elevation of ammonia can be hypothesized as follows: First, the lack of acetyl-CoA resulted from ineffective transportation of

long chain fatty acids into mitochondria for beta-oxidation (Walser and Stewart 1981). Acetyl-CoA is the precursor of N-acetylglutamate (NAG) synthesis, the co-factor of urea cycle. Therefore, the urea cycle cannot function normally to transform ammonia to urea. This patient also experienced intermittently prolonged fasting states during the religious Ramadan fasting month which can contribute to a lack of energy source and acetyl-coA. Secondly, the patient consumed large amount of skin-on chicken which has a large amount of fat content and long chain fatty acids that cannot be used in patients with *CPTII* deficiency. The long chain fatty acids are detoxified by carnitine but that may lead to carnitine insufficiency and worsen the mitochondrial functions (Limketkai and Zucker 2008). Thirdly, the patient received a large loading dose of valproate when he had seizure on top of daily valproate he had received for his behavioral control. Valproate was reported to cause fatal hyperammonemia (Bega et al. 2012). The mechanism of valproate toxicity is believed to be the inhibition of the NAG synthase, the beginning of the urea cycle (Aires et al. 2011). This might worsen hyperammonemia in our patient. In contrast to previously reported patients with *CPTII* deficiency with hyperammonemia, our patient did not have liver dysfunction as the cause of his marked elevation of ammonia (Ikeda et al. 2017; Malik et al. 2015).

Our patient has had recurrent episodes of epilepsy or encephalopathy with or without hyperammonemia. It is possible that some hyperammonemic occurrences were spurious; the collection method and transport are known to interfere with the measurement of serum ammonia level. The false association of high protein content food and the effect on symptoms with sodium benzoate led to the diagnosis of urea cycle disorder prior to the molecular

Table 1 Plasma acylcarnitine profile consistent with CPTII deficiency

Acylcarnitine	Result (μmol/L)	Reference range (μmol/L)
C0	3.22	5.55–20.88
C4	0.05	0.04–0.82
C6	0.03	0.02–0.16
C8	0.09	0.02–0.77
C10	0.46	0.03–0.87
C12	0.14	0.01–0.25
C14	0.14	0.00–0.11
C16	0.86	0.03–0.22
C16:1	0.21	0.00–0.09
C16:1OH	0.01	0.00–0.05
C18	0.19	0.01–0.13
C18:1	0.77	0.02–0.38
C18:1OH	0.01	0.00–0.05

confirmation. Food with high protein content usually has large amount of fat and fatty acid, such as skin-on chicken with oily-cooked rice. One time he developed symptoms after consuming a large piece of salmon, one of the highest fat content fish (Cladis et al. 2014). Plasma amino acid analysis in this case did not reveal specific enzymatic defect in urea cycle. The low levels of citrulline, argininosuccinate, and ornithine suggest N-acetyl glutamate deficiency or carbamoyl phosphate synthetase I deficiency (Caldovic et al. 2010) but generalized low amino acid levels reflect his nutritional status and protein restriction prior to being tested. Plasma acylcarnitine profile was not performed as a routine biochemical work up at the time of initial evaluation which was more than two decades ago. We collected and sent the plasma acylcarnitine profile after the molecular diagnosis of CPTII. It is consistent with CPTII deficiency (Table 1) with markedly elevation of C14, C16, C16:1, C18 and C18:1 (bold).

Patients with homozygous p.L644S mutation have not previously been reported in the literature. However, a 6-month-old suddenly unexpectedly died was found to be compound heterozygous for p.L644S and p.F388Y (Yamamoto et al. 2011). Postmortem analysis and plasma acylcarnitine profile suggestive of fatty acid oxidation disorder were consistent with the diagnosis of CPTII deficiency. The majority of Caucasian patients with the most common adult myopathic form carry at least one allele of p.S113 L (Joshi et al. 2014; Shima et al. 2016; Anichini et al. 2011; Thuillier et al. 2003; Fanin et al. 2012). Null alleles have been reported in several lethal neonatal form (Vatanavicharn et al. 2015). Patient with severe hepatocardiomyocardial infantile form developed symptoms or signs in the first year of life. CPTII deficiency is one of the quite not uncommon causes of sudden unexpected infantile death (Yamamoto et al. 2011; Bouchireb et al. 2010; Meir et al. 2009; Takahashi et al.

2016). However, many reports showed that these patients can survive through teenage or later with the specific dietary management and the supplement of medium chain triglycerides or triheptanoin (Taroni et al. 1992; Wataya et al. 1998; Martinez 1997; Roe et al. 2008). Our patient survived his second decades without specific treatment for his newly diagnosed fatty acid oxidation disorder suggesting a milder phenotype associated with this homozygous mutation. Intermediate form is not described in the literature though. Patients with myopathic form do not show recurrences episodes of hypoglycemia, cardiomyopathy, or liver damage but rhabdomyolysis (Anichini et al. 2011; Joshi et al. 2014). Our patient's older sister was died at the age of 1 year with epileptic encephalopathy and it is presumed that she might be suffering from the same condition, but this cannot be proved. Interfamilial variation and difference in severity in patients with the same mutations might reflect the environment and epigenetic modifying factors.

Conclusion

We report here a patient with severe hyperammonemic encephalopathy requiring dialysis removal of ammonia. He carries a homozygous p.L644S mutation in *CPTII*. This presentation has not been reported before in patients with CPTII deficiency. The aggravating factors were thought to be prolonged fasting during the Ramadan month, the high fat consumption, and the effects of valproate. Fatty acid oxidation disorders should be in the differential diagnosis of hyperammonemia in patients suggestive of metabolic disorders such as intermittent symptoms associated with exaggerated fasting or feeding states.

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Synopsis

Long chain fatty acid oxidation disorders should be considered in the differential diagnosis of hyperammonemia even without liver dysfunction.

Contributions of Individual Authors

Prasit Phowthongkum planned and prepared the report and was a medical geneticist consulted on this patient during the critical episode described in this manuscript.

Chupong Ittiwut analyzed the whole exome sequencing, was responsible heavily for the accuracy of the methods and filtering criteria described, and reviewed the final manuscript.

Vorasuk Shotelersuk was responsible for the authorization of the whole exome sequencing result, was a medical geneticist who followed this patient from the early manifestation in childhood, and reviewed the manuscript, commented, and made a final decision to submit the manuscript after proofread.

Guarantor of This Manuscript

Prasit Phowthongkum.

Declaration of Competing Interest

Prasit Phowthongkum, Chupong Ittiwut, and Vorasuk Shotelersuk have no competing interest to be declared financially or non-financially related to the submission of this manuscript.

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Ethics Approval

This case report has been waived for the ethics approval by the standard of the Institutional Research Board of the Faculty of Medicine, Chulalongkorn University and all procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000 (5).

Consent

The guardian of the patient gave the informed consent to publish this case and personal information. The consent form is available upon request.

References

- Aires CC, van Cruchten A, Ijlst L, de Almeida IT, Duran M, Wanders RJ, Silva MF (2011) New insights on the mechanisms of valproate-induced hyperammonemia: inhibition of hepatic N-acetylglutamate synthase activity by valproyl-CoA. *J Hepatol* 55:426–434
- Anichini A, Fanin M, Vianey-Saban C, Cassandrini D, Fiorillo C, Bruno C, Angelini C (2011) Genotype-phenotype correlations in a large series of patients with muscle type CPT II deficiency. *Neurol Res* 33:24–32
- Bega D, Vaitkevicius H, Boland TA, Murray M, Chou SH (2012) Fatal hyperammonemic brain injury from valproic acid exposure. *Case Rep Neurol* 4:224–230
- Boemer F, Deberg M, Schoos R, Caberg JH, Gaillez S, Dugauquier C et al (2016) Diagnostic pitfall in antenatal manifestations of CPTII deficiency. *Clin Genet* 89:193–197
- Bonnefont JP, Djouadi F, Prip-Buus C, Gobin S, Munnich A, Bastin J (2004) Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. *Mol Aspects Med* 25:495–520
- Bouchireb K, Teychene AM, Rigal O, de Lonlay P, Valayannopoulos V, Gaudelus J et al (2010) Post-mortem MRI reveals CPTII deficiency after sudden infant death. *Eur J Pediatr* 169:1561–1563
- Caldovic L, Mew NA, Shi D, Morizono H, Yudkoff M, Tuchman M (2010) N-acetylglutamate synthase: structure, function and defects. *Mol Genet Metab* 100:S13–S19
- Cladis DP, Kleiner AC, Freiser HH, Santerre CR (2014) Fatty acid profiles of commercially available finfish filets in the United States. *Lipids* 49:1005–1018
- Fanin M, Anichini A, Cassandrini D, Fiorillo C, Scapolan S, Minetti C et al (2012) Allelic and phenotypic heterogeneity in 49 Italian patients with the muscle form of CPT-II deficiency. *Clin Genet* 82:232–239
- Hung TY, Chen CC, Wang TL, Su CF, Wang RF (2011) Transient hyperammonemia in seizures: a prospective study. *Epilepsia* 52:2043–2049
- Ikeda N, Maruyama S, Nakano K, Imakiire R, Ninomiya Y, Seki S et al (2017) A surviving 24-month-old patient with neonatal-onset carnitine palmitoyltransferase II deficiency. *Mol Genet Metab Rep* 11:69–71
- Joshi PR, Deschauer M, Zierz S (2014) Carnitine palmitoyltransferase II (CPT II) deficiency: genotype-phenotype analysis of 50 patients. *J Neurol Sci* 338:107–111
- Limketkai BN, Zucker SD (2008) Hyperammonemic encephalopathy caused by carnitine deficiency. *J Gen Intern Med* 23:210–213
- Malik S, Padiwal AA, Korday CS, Jadhav SS (2015) Neonatal carnitine palmitoyltransferase II deficiency: a lethal entity. *J Clin Diagn Res* 9:SD01–SD02
- Martinez GR (1997) Favourable clinical evolution in a 20-year old girl with the infantile form of carnitine palmitoyltransferase II deficiency. In: 7th international congress of inborn errors of metabolism, p. Abstr O101, Vienna
- Meir K, Fellig Y, Meiner V, Korman SH, Shaag A, Nadjari M, Soffer D, Ariel I (2009) Severe infantile carnitine palmitoyltransferase II deficiency in 19-week fetal sibs. *Pediatr Dev Pathol* 12:481–486
- Roe CR, Yang BZ, Brunengraber H, Roe DS, Wallace M, Garritson BK (2008) Carnitine palmitoyltransferase II deficiency: successful anaplerotic diet therapy. *Neurology* 71:260–264
- Shima A, Yasuno T, Yamada K, Yamaguchi M, Kohno R, Yamaguchi S et al (2016) First Japanese case of carnitine palmitoyltransferase II deficiency with the homozygous point mutation S113L. *Intern Med* 55:2659–2661
- Sigauke E, Rakheja D, Kitson K, Bennett MJ (2003) Carnitine palmitoyltransferase II deficiency: a clinical, biochemical, and molecular review. *Lab Invest* 83:1543–1554
- Takahashi Y, Sano R, Kominato Y, Kubo R, Takahashi K, Nakajima T et al (2016) A case of sudden unexpected infant death involving a homozygotic twin with the thermolabile CPTII variant, accompanied by rotavirus infection and treatment with an antibiotic containing pivalic acid. *Legal Med* 22:13–17
- Taroni F, Verderio E, Fiorucci S, Cavadini P, Finocchiaro G, Uziel G, Lamantea E, Gellera C, DiDonato S (1992) Molecular characterization of inherited carnitine palmitoyltransferase II deficiency. *Proc Natl Acad Sci* 89:8429–8433

- Thuillier L, Rostane H, Droin V, Demaugre F, Brivet M, Kadhon N et al (2003) Correlation between genotype, metabolic data, and clinical presentation in carnitine palmitoyltransferase 2 (CPTII) deficiency. *Hum Mutat* 21:493–501
- Vatanavicharn N, Yamada K, Aoyama Y, Fukao T, Densupsoontorn N, Jirapinyo P et al (2015) Carnitine-acylcarnitine translocase deficiency: two neonatal cases with common splicing mutation and in vitro bezafibrate response. *Brain Dev* 37:698–703
- Walser M, Stewart PM (1981) Organic acidaemia and hyperammonaemia: review. *J Inherit Metab Dis* 4:177–182
- Wataya K, Akanuma J, Cavadini P, Aoki Y, Kure S, Invernizzi F et al (1998) Two CPT II mutations in three Japanese patients with carnitine palmitoyltransferase II deficiency: functional analysis and association with polymorphic haplotypes and two clinical phenotypes. *Hum Mutat* 11:377–386
- Yamamoto T, Tanaka H, Kobayashi H, Okamura K, Tanaka T, Emoto Y et al (2011) Retrospective review of Japanese sudden unexpected death in infancy: the importance of metabolic autopsy and expanded newborn screening. *Mol Genet Metab* 102:399–406