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Case Report

Clinical and molecular characteristics of carnitine-acylcarnitine translocase deficiency with c.270delC and a novel c.408C>A variant

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ABSTRACT

Background. Carnitine-acylcarnitine translocase deficiency (CACTD) is a rare, autosomal recessive, and highly lethal fatty acid oxidation (FAO) disorder caused by defective acylcarnitine transport across the mitochondrial membrane. CACTD is characterized by severe episodes of hypoglycemia and hyperammonemia, seizures, cardiomyopathy, liver dysfunction, severe neurological damage, and muscle weakness. Herein, we described the clinical features, biochemical, and molecular findings of three patients with CACTD, presented with poor feeding, hypoglycemia, liver dysfunctions, and hyperammonemia, but died despite intensive treatment.

Cases. All cases had similar signs and symptoms like poor feeding and respiratory failure associated with liver dysfunction. Urinary organic acid profiles in the presence of hypoglycemia and hyperammonemia led us to the possible diagnosis of one of fatty acid β-oxidation defects. Results of the molecular analyses were compatible with CACTD. In addition to known mutation (c.270delC;p.Phe91Leufs*38) we detected a novel one (c.408C>A;p.Cys136*).

Conclusions. All three cases died despite a very intensive therapy. Based on our experience with these three cases, it can be said that CACTD has a relatively poor prognosis, molecular studies are of most importance in suspected cases for the final diagnosis and such studies might be of help while giving genetic counselling and guidance to parents for future pregnancies.

Key words: fatty acid oxidation defects, carnitine, aclycarnitines, carnitine acyltransferases, carnitine acylcarnitine translocase.

Fatty acid β-oxidation (FAO) to acetyl coenzyme A in the mitochondrial matrix requires an energy-yielding pathway controlled by specific enzymes.[1] This energy pathway is crucial for cardiac and skeletal muscles during long-term exercise and prolonged fasting.[2] The transfer of acyl-coenzyme As (acyl-CoAs) through the mitochondrial membrane needs L-carnitine, two types of carnitine palmitoyl transferases (CPT I and II), and carnitine-acylcarnitine translocase (CACT), which is a critical enzyme in the carnitine cycle.[3] Carnitine-acylcarnitine translocase deficiency (CACTD) is a rare, autosomal recessive and highly lethal FAO-disorder caused by defective acylcarnitine transport across the mitochondrial membrane, and characterized by severe episodes of hypoglycemia and hyperammonemia, seizures, cardiomyopathy, liver dysfunction, severe neurological damage, and muscle weakness.[2,4,5] Most CACTD cases have developed extreme metabolic decompensation and unexplained early death in the first year of their lives.[6]

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The gene encoding CACT (*SLC25A20*) consists of nine exons, spans about 16.5 kb, and is located on chromosome 3p21.31.[7,8] At least 42 different pathogenic/possible pathogenic mutations, including 20 missense/nonsense mutations, 10 small deletions, 2 small insertions, 1 small indel, 4 gross deletions, and 5 splicing mutations have been reported in this gene.[6] The frequently reported variation in patients with CACTD is c.199-10T>G splice site mutation in Asian population.[5,6,9] No patients with CACTD have previously been reported from Turkey.

In this study, we aimed to describe the clinical, biochemical, and molecular features of three CACTD patients.

Case Reports

Three patients followed up with a diagnosis of CACTD were reported. The patients' data were retrieved from the hospital's electronic patient registry (Nucleus Automation System) and patient files.

Genetic investigations

Genomic DNA was isolated from peripheral blood samples (10 ml) using a standard salting-out method. Whole exomes sequencing (WES) was performed on patient 1 and WES data was evaluated by bioinformatics analysis. We filtered variants according to their minor allele frequency lower than 1% within different open access population allele frequency databases (1000 Genomes Project and dbSNP138). Then, exonic and non-synonymous variants were retrieved. We removed variants that were already found in our in-house variant database. Variants present in the 'Genome Aggregation Database Browser' (gnomAD) as homozygous state were ruled out. The novel homozygous variant in the *SLC25A20* gene was visually inspected using Integrative Genomics Viewer for patient 1. This novel variant was further confirmed and familial segregation analysis was performed by Sanger sequencing. On the other hand, the gene panel including around 450 genes accounting for inborn errors of metabolism was performed in patient 2 and patient 3. Using this panel, detected nucleotide changing was also verified by Sanger sequencing in the *SLC25A20* gene for patients 2 and 3.

All three patients were girls, born to young mothers following 37-39 gestational weeks, and presented with poor feeding in the first few days of life. The most remarkable clinical findings of all patients are summarized in Table I.

Patient 1

This female neonate was the third child of consanguineous parents whose second baby died 6w postpartum. Her birth weight was 2,630 g. The patient was hospitalized because of respiratory failure and poor feeding on the second day of life. Her laboratory findings showed hypoglycemia (20 mg/dl; normal range: 70-130 mg/dl), hyperammonemia (1019 mmol/L; normal value ≤110mmol/L), elevated LDH (656 IU/L) and transaminases (ALT 168 IU/L, AST 194 IU/L). LC-MS/MS analysis of her dried blood sample showed increased levels of long-chain acylcarnitines, particularly C_{16}, $C_{16.1}$, C_{18}, $C_{18.1}$ acylcarnitines, and decreased concentrations of C_0 free carnitine.

Her diet was adjusted to include medium-chain triglycerides (MCT) and carbohydrates, limiting long-chain fatty acids. The blood carnitine levels before and after carnitine treatments are shown in Table II. Additionally, the urinary organic acid analysis demonstrated elevated lactic acid (0.5-fold), and an increased urinary excretion of 3-OH isovaleric acid (0.5-fold), adipic acid (1.3-fold), suberic acid (2.5-fold), and sebacic acid (2.5-fold). Mutation analyses in *SLC25A20* gene (NM_000387.6) revealed a novel homozygous c.408C>A(p.Cys136*) nucleotide change. This nucleotide changing c.408C>A (p.Cys136*) in exon 4 in the *SLC25A20* gene was homozygous in patient 1 (II-3) and heterozygous in the parents (I-1 and I-2) and healthy sibling (II-1) (Figs 1A and 1B). The patient was given a special diet containing 20-25% fat, and supplemented with multivitamins, and oral carnitine 30 mg/kg/day, and advised to avoid long fasting periods. The patient was discharged on day 16, but she died at 10 months of life.

Table I. Characteristics of CACTD patients.

	Patient 1	Patient 2	Patient 3
Age of mother (year)	26	32	31
Sex	Female	Female	Female
Birth weight (g)	2,630	3,480	3,540
Gestational age (week)	37	37	39
Parental consanguinity	Present	Absent	Absent
Previous deceased siblings	First child	One sister died	Two brothers died
Day of onset	2nd day of life	1st day of life	10th day of life
Signs and symptoms	Poor feeding, respiratory failure	Poor feeding, respiratory failure	Poor feeding
Age at death	10 months	12 months	52 days
Genotype	Homozygous c.408C>A (p.Cys136*)	Homozygous c.270delC (p.Phe91Leufs*38)	Homozygous c.270delC (p.Phe91Leufs*38)
ALT (IU/L)	168	59	80
AST (IU/L)	194	68	147
LDH (IU/L)	656	452	502
Ammonia (mmol/L)	1019	834	1025

ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase.

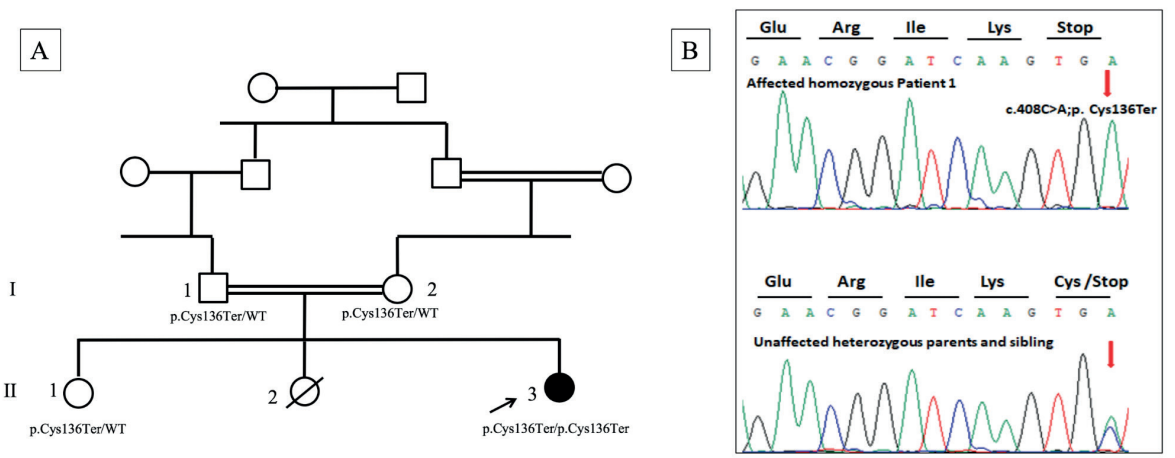

Fig. 1. Pedigree of Patient 1 (A), and Sanger sequencing of p.Cys136* (c.408C>A) variant in *SLC25A20* gene (NM_000387.6) among family members (B).

Patient 2

This baby girl was born to non-consanguineous parents after a normal gestation and delivery, weighing 3,480 g at birth. Her older sister had reportedly died two months after birth because of a suspected but remained undiagnosed metabolic disease. She presented on the first day of life with poor feeding, respiratory failure, hypotonia, hypoglycemia (20 mg/dl) and hyperammonemia (834 mmol/L). The results of LC-MS/MS analysis of a dried blood sample showed increased levels of long-chain acylcarnitines, particularly C_{16} acylcarnitine, and decreased concentrations of C_0 free carnitine (Table II).

Furthermore, the urinary organic acid analysis showed highly elevated levulinic acid, oxalic acid, fumaric acid, suberic acid, and adipic acid, whereas mildly elevated methylmalonic

Table II. Acylcarnitine concentrations in dried blood spots before and after low dose L-carnitine supplements treatment.

Acylcarnitine (mmol/L)	Patient 1		Patient 2		Patient 3		Normal range
	Before	After	Before	After	Before	After	
C_0 (free carnitine)	4.39	11.89	4.23	8.65	3.10	15.85	10-60
C_{14} (myristoil carnitine)	0.45	0.47	0.66	1.86	0.44	0.99	0-0.36
C_{16} (palmitoil carnitine)	8.04	6.26	12.87	8.94	2.62	3.94	0-1.51
$C_{16.1}$ (palmitoil carnitine)	0.52	0.61	0.95	0.86	0.33	0.60	0-0.27
C_{18} (steraoil carnitine)	1.41	1.5	1.38	1.31	0.78	0.99	0-0.61
$C_{18.1}$ (oleil carnitine)	1.92	1.78	4.01	3.27	2.44	2.72	0-1.51

acid, 3-OH sebacic acid, dodecanedioic acid, and 5-OH hexanoic acid excretions. Feeding was initiated with a diet rich in MCT and carbohydrates, limiting long-chain fatty acids. The patient received oral carnitine (30 mg/kg/day). A homozygous c.270delC deletion (p.Phe91Leufs*38) was detected in the *SLC25A20* gene (NM_000387.6). Following supportive treatment and dietary adjustment, the patient was discharged on day 42; however, she died at 12 months of life.

Patient 3

This female neonate, the third child of healthy non-consanguineous parents, was born at full term. Her birth weight was 3,540 g. Her older two brothers had died on the second and fifth days of their lives respectively due to unknown causes.

In the first ten days of life, the patient was followed up with suspected diagnosis of neonatal hepatitis. After ten days, when she stopped receiving breast feeding, and started to vomit, hepatomegaly and hyperammonemia were noted. Her serum ALT, AST, and LDH levels were 80, 147, and 502 IU/L, respectively. Blood ammonia was measured as 1025 mmol/L. LC-MS/MS analysis of her dried blood sample yielded decreased concentrations of C_0 free carnitine and increased concentrations of C_{14}, C_{16}, $C_{16.1}$, C_{18}, and $C_{18.1}$ (Table II). Additionally, the urinary organic acid analysis showed elevated adipic acid (2/3-fold), sebacic acid (1.5-fold), 3-OH sebacic acid (2/3-fold), and 3-OH dodecanedioic acid (1/6-fold) excretions. Mutation analysis of the *SLC25A20* gene revealed a homozygous c.270delC (p.Phe91Leufs*38) deletion. Despite all supportive measures and dietary adjustment (rich in MCT and carbohydrates, limiting long-chain fatty acids), the patient died at on the 52nd day of life.

Ethical approval for this study was obtained from Hacettepe University Faculty of Medicine, Clinical Research Ethics Committee (GO: 20/530-2020/11-39).

The parents of the patient were informed, and written and oral consent was obtained according to the principles of the Helsinki Declaration.

Discussion

CACTD is a rare inborn disorder of the carnitine cycle that is highly lethal before one year of age.[10] More than 60 patients have been reported worldwide during the last two decades.[5] The clinical features of this disorder are generally resulted from a combination of energy deprivation and endogenous toxicity due to accumulation of long-chain acylcarnitines.[11] Brain, liver, muscle, and heart are the primarily affected organs in this disorder which accounts for CACTD patients suffering from neurological abnormalities, muscle damage, cardiomyopathy, and liver dysfunctions.[12]

In this report, we presented three CACTD patients, all of whom had similar features, including liver dysfunctions, respiratory failure, and poor feeding. Presence of hypoglycemia and hyperammonemia along with dicarboxylic

aciduria in urinary organic acid and LC-MS/MS results led us to consider defects in fatty acid β-oxidation. As in previously reported cases, our patients had high blood levels of ammonia, ALT, AST, LDH, highly elevated C_{16}, $C_{16.1}$ carnitine esters, moderately elevated C_{18} carnitine esters, and low concentrations of C_0 free carnitine.[1-3,5,6,11] The diets given, treatment options, biochemical and clinical features were alike in all three CACTD patients. Unfortunately, their life spans were limited despite supportive care efforts.

Genetic mutation analyses of the *SLC25A20* gene showed that Patient 1, 2, and 3 have homozygous mutations: a novel c.408C>A (p.Cys136*) nonsense mutation, which has not been reported yet and a previously-reported c.270delC (p.Phe91Leufs*38) frameshift mutation.[13,14] Previously reported cases indicate that c.270delC mutation might be associated with severe phenotype in patients.[11,15] In this study, patients 2 and 3, who had c.270delC mutations showed a severe phenotype that gives further support to this assumption. These two patients died on the 12th month and 52nd day of life respectively. Carnitine-acylcarnitine translocase protein which belongs to the mitochondrial carrier family consist of 6 transmembran α-helices (two in each repetitive domain) domains. Previously reported in consanguineous Turkish family as pathogenic variant c.306delC in exon 3 frameshift mutation give rise to the stop codon at amino acid residue 127 which is predicted to cause premature protein truncation. This mutant protein that would lack the second and third transmembrane domains and would presumably result in nonfunctional translocase enzyme.[13]

Patient 1, who had a novel homozygous c.408C>A (p.Cys136*) nonsense mutation, had also severe phenotype, and she died on the 10th month of her life. This truncating mutation is predicted to occur in the second intra mitochondrial hydrophilic loop and resulting protein would lack fourth, five and sixth transmembrane domains and would therefore be completely inactive. Hence, our data further reinforce that the c.270delC (p.Phe91Leufs*38) mutation is associated with a severe phenotype of CACTD and a novelc.408C>A (p.Cys136*) variation has the potential to be associated with severe phenotype too.

In this paper, we reported three cases of CACTD. Although significant progress in early recognition and appropriate treatment is crucial and has been made according to clinical signs and symptoms developing shortly after birth, even with expanded newborn screening, including detecting urinary organic acid excretions, and C_0, C_{16}, $C_{16.1}$, C_{18}, and $C_{18:1}$ acylcarnitines in dried blood spots, this disorder has still a high mortality rate.[16,17] On the other hand, apart from the frameshift mutation c.270delC, we identified a novel c.408C>A nonsense mutation. Almost all patients with CACTD who had a genotype of those mutations present with severe clinical phenotypes. The available evidence clearly shows that CACTD is generally a highly severe and lethal metabolic disorder with clinical, biochemical, and molecular heterogeneity. Although CACTD causes early neonatal death, molecular diagnosis is quite imperative for providing better and effective reproductive guidance for future pregnancies.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BBG, AT, AD, HSS, TC; data collection: BBG, CK, DYY; analysis and interpretation of results: BBG, DYY, CK, RKÖ; draft manuscript preparation: BBG, AT, TC. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors have no conflict of interest in this study.

REFERENCES

1. Galron D, Birk OS, Kazanovitz A, Moses SW, Hershkovitz E. Carnitine-acylcarnitine translocase deficiency: identification of a novel molecular defect in a Bedouin patient. J Inherit Metab Dis 2004; 27: 267-273.
2. Yan HM, Hu H, Ahmed A, et al. Carnitine-acylcarnitine translocase deficiency with c.199-10 T>G and novel c.1A>G mutation: two case reports and brief literature review. Medicine (Baltimore) 2017; 96: e8549.
3. Iacobazzi V, Invernizzi F, Baratta S, et al. Molecular and functional analysis of SLC25A20 mutations causing carnitine-acylcarnitine translocase deficiency. Hum Mutat 2004; 24: 312-320.
4. Iacobazzi V, Pasquali M, Singh R, et al. Response to therapy in carnitine/acylcarnitine translocase (CACT) deficiency due to a novel missense mutation. Am J Med Genet A 2004; 126A: 150-155.
5. Vitoria I, Martín-Hernández E, Peña-Quintana L, et al. Carnitine-acylcarnitine translocase deficiency: experience with four cases in Spain and review of the literature. JIMD Rep 2015; 20: 11-20.
6. Tang C, Liu S, Wu M, et al. Clinical and molecular characteristics of carnitine-acylcarnitine translocase deficiency: experience with six patients in Guangdong China. Clin Chim Acta 2019; 495: 476-480.
7. Yang BZ, Mallory JM, Roe DS, et al. Carnitine/acylcarnitine translocase deficiency (neonatal phenotype): successful prenatal and postmortem diagnosis associated with a novel mutation in a single family. Mol Genet Metab 2001; 73: 64-70.
8. Iacobazzi V, Naglieri MA, Stanley CA, Wanders RJ, Palmieri F. The structure and organization of the human carnitine/acylcarnitine translocase (CACT1) gene2. Biochem Biophys Res Commun 1998; 252: 770-774.
9. Vatanavicharn N, Yamada K, Aoyama Y, et al. Carnitine-acylcarnitine translocase deficiency: two neonatal cases with common splicing mutation and in vitro bezafibrate response. Brain Dev 2015; 37: 698-703.
10. Lopriore E, Gemke RJ, Verhoeven NM, et al. Carnitine-acylcarnitine translocase deficiency: phenotype, residual enzyme activity and outcome. Eur J Pediatr 2001; 160: 101-104.
11. Rubio-Gozalbo ME, Bakker JA, Waterham HR, Wanders RJ. Carnitine-acylcarnitine translocase deficiency, clinical, biochemical and genetic aspects. Mol Aspects Med 2004; 25: 521-532.
12. Indiveri C, Iacobazzi V, Tonazzi A, et al. The mitochondrial carnitine/acylcarnitine carrier: function, structure and physiopathology. Mol Aspects Med 2011; 32: 223-233.
13. Hsu BY, Iacobazzi V, Wang Z, et al. Aberrant mRNA splicing associated with coding region mutations in children with carnitine-acylcarnitine translocase deficiency. Mol Genet Metab 2001; 74: 248-255.
14. Costa C, Costa JM, Slama A, et al. Mutational spectrum and DNA-based prenatal diagnosis in carnitine-acylcarnitine translocase deficiency. Mol Genet Metab 2003; 78: 68-73.
15. Huizing M, Wendel U, Ruitenbeek W, et al. Carnitine-acylcarnitine carrier deficiency: identification of the molecular defect in a patient. J Inherit Metab Dis 1998; 21: 262-267.
16. Morris AA, Olpin SE, Brivet M, Turnbull DM, Jones RA, Leonard JV. A patient with carnitine-acylcarnitine translocase deficiency with a mild phenotype. J Pediatr 1998; 132 (3 Pt 1): 514-516.
17. Ogawa A, Yamamoto S, Kanazawa M, Takayanagi M, Hasegawa S, Kohno Y. Identification of two novel mutations of the carnitine/acylcarnitine translocase (CACT) gene in a patient with CACT deficiency. J Hum Genet 2000; 45: 52-55.