ARC Journal of Nephrology
Volume 5, Issue 1, 2020, PP21-27
www.arcjournals.org

A Case of Myoglobin Cast Nephropathy with a Variant of Homozygote Mutation in Carnitinepalmitoy ltransferase2 (CPT2) Gene

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Abstract: *Rhabdomyolysis is a clinical syndrome ranging from myalgia to acute kidney injury. Trauma, use of drugs such as cocaine, amphetamines, statins, heroin etc, extremes of body temperature, seizures or muscle tremors, severe dehydration, severe exertion, such as marathon running or calisthenics, genetic muscle diseases such as Carnitine palmitoyltransferase (CPT) II deficiency, etc cause rhabdomyolysis and acute kidney injury. CPT II is an enzyme that works in muscle fatty acid metabolism. Deficiency of CPT II is characterized one of the reasons of rhabdomyolysis without persistent muscle weakness and lipid accumulation in muscle fibers. The biochemical consequences of the disease-causing mutations are still discussed controversially. We present a case with CPT II gene mutation that manifested with cast nephropathy, rhabdomyolysis and AKI.*

Keywords: *Carnitine palmitoyltransferase 2 deficiency, mutation, rhabdomyolysis, acute kidney injury*.

1. INTRODUCTION

Rhabdomyolysis is a serious and potentially life threatening syndrome characterized by muscle necrosis and the release of intracellular muscle constituents into the circulation. The clinical presentation varies from classical symptoms such as myalgia and weakness to acute kidney injury (AKI) (1). The acquired causes of rhabdomyolysis have been identified in three categories as traumatic or muscle compression (crush syndrome, prolonged immobilization, vascular and orthopaedic surgeries), non-traumatic exertional or non-exertional. Hereditary causes are genetic disorders associated with terminal glycolysis or glycogenolysis, lipid metabolism, crebs cycle, mitochondrial respiratory chain, myopathies and others. Furthermore, some of the acquired causes (infections, exercise or medication) can trigger rhabdomyolysis in patients with underlying hereditary metabolic disorders (2-4) Here we present a male patient with carnitine palmitoyltransferase type 2 (CPT2) deficiency who had a homozygous missense mutation (c.338C>T/p.Ser113Leu) on the CPT2 (NM_000098) gene.

2. CASE REPORT

A twenty-two-year-old male patient applied to our emergency department with complaints of nausea, vomiting, abdominal pain, weakness and dark red discoloration in his urine. The patient, who did not have any obvious complaints before, stated that his brother was treated in the hospital with similar complaints at the same ages. There was no feature in his physical examination. In laboratory tests; urea 282 mg / dL, creatinine 10.9 mg / dL, uric acid 12.6 mg / dL, total protein 7.2 g / dL, albumin 3.8 g / dL, total bilirubin 0.41 mg / dL, direct bilirubin 0.1 mg / dL, total cholesterol 106 mg / dL, triglyceride 240 mg / dL, low density lipoprotein 34 mg / dL, creatinine phosphokinase 36,680 U / L, aspartate aminotransferase (AST) 2044 IU / L, alanine aminotransferase (ALT) 840 IU / L, alkaline phosphatase 58 IU / L, gamma glutamil transferase 28 IU / L, calcium 8.0 mg / dL , phosphorus 9.0 mg / dL, sodium 131 mEq / L, potassium 5.0 mg / dL, chlorine 93 mEq / l, leukocyte 6930 / mm3, hemoglobin 12 g / dL, platelet 175,000 / mm3, free T3 2.01 pg / mL, free T4 1.23 ng / dL, thyroid stimulating

hormone 1.45 IU / mL, parathyroid hormone 304.2 pg / mL, erythrocyte sedimentation rate 47 mm / h, and high sensitive C reactive protein (CRP) 43.1 mg / L detected. Anti-Hepatitis C antibody, anti- Human Immunodeficiency Virus and Hepatitis B surface antigen were negative. In urine analysis; density 1014, pH 6.0, glucose (-), protein +++ positive detected and sediment analysis showed 3 leukocytes, 5 red cells and leukocyte cylinders in HPF. Daily protein excretion was 3.3 g/day. On renal ultrasonography, both kidney sizes were 118 mm, parenchymal thicknesses were 16 mm, and parenchyma echogenities increased in grade II-III. The patient hospitalized with the diagnosis of acute kidney injury. Kidney biopsy revealed 9 glomeruli, two of them were global sclerotic. There was hypertrophic appearance in the glomeruli. No pathology observed in the mesangial area and basement membranes. Chronic inflammation, peritubular capillaryitis, including exonophils, found in the interstitial space. Brown pigment deposits present in the tubule lumens in the medulla (Figure 1). Immunohistochemical examination revealed positive immunoreactivity with pigmented myoglobin. (Figure 1D). No features sew in the arteriols. Prussian blue and Congo staining were negative. In immunofluorescence microscopy, Immunoglobulin (Ig) G, IgA, IgM, complement (C) 3, fibrinogen, C1q, kappa and lambda were negative. No immunocomplex nephritis detected in immunofluorescence examination (Figure 1). Myoglobin pigment detected in the tubular lumens, it was suggested to investigate the patient for the etiology of myoglobinuria. After dialysis (5 sessions) and medical treatment, the patient recovered and discharged. In his last tests, creatinine measured 0.98 mg/dL. Urea and creatinine were determined higher in the patient's brother who admitted to the cardiology service 5 years ago, at the age of 23 with dyspnea and abdominal pain. In the examinations of that period; urea 128 mg / dL, creatinine 10.83 mg / dL, potassium 4.7 mEq / L, sodium 131 mEq / L, calcium 8.2 mg / dL, phosphorus 5.8 mg / dL, magnesium 2.55 mg / dL lactate dehydrogenase 712 U / L, AST 84 U / L, ALT 46 U / L, hemoglobin 13.1 g / dL, leukocyte 14,200 / mm3, platelet 212,000 / mm3, high-sensitive CRP 71.8 mg / L. In renal ultrasonography, minimal fluid on the right of perirenal area found. The patient diagnosed with acute kidney injury due to rhabdomyolysis. Kidney biopsy performed with the diagnosis of rapidly progressive glomerulonephritis but myoglobin cylinder nephropathy detected.

The patient discharged when he recovered completely after dialysis and medical treatment. When the family tree of the patient evaluated; the grandfather had two healthy boys. The grandfather had one daughter, three brothers and their children. In her third uncle, one boy and one girl had mental retardation. He had three uncles and two of them were lost at an early age due to unknown reasons. (Figure 2). Four children (three girls, one boy) of their living uncles were healthy. Renal functions and muscle enzymes were normal in both patients at their last examination.

2.1. Renalbiopsy

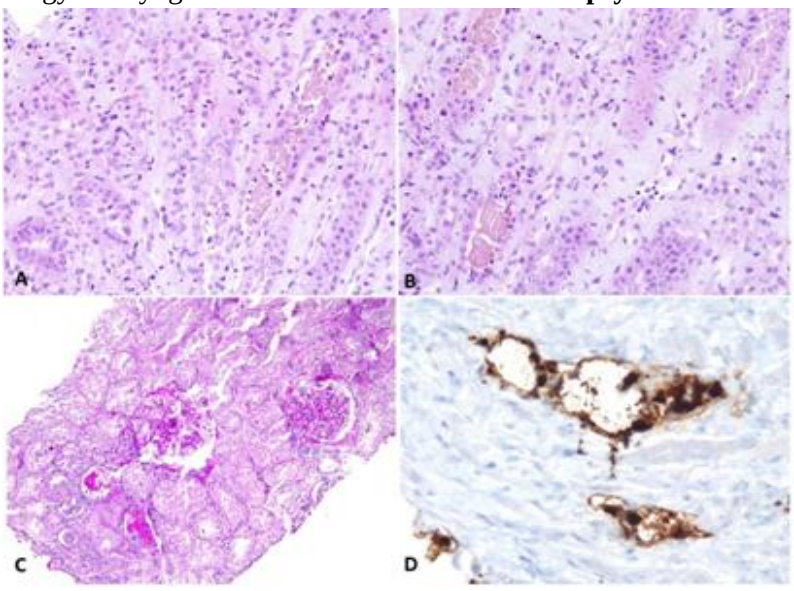


Figure1: *Pigmented granular cylinders (A-B) in tubulus lumens, normal appearance in glomeruli (C), and strong positive immunoreactivity with Myoglobin in tubular cylinders (D) [A-B HE, C PAS, D anti-Myoglobin antibody].*

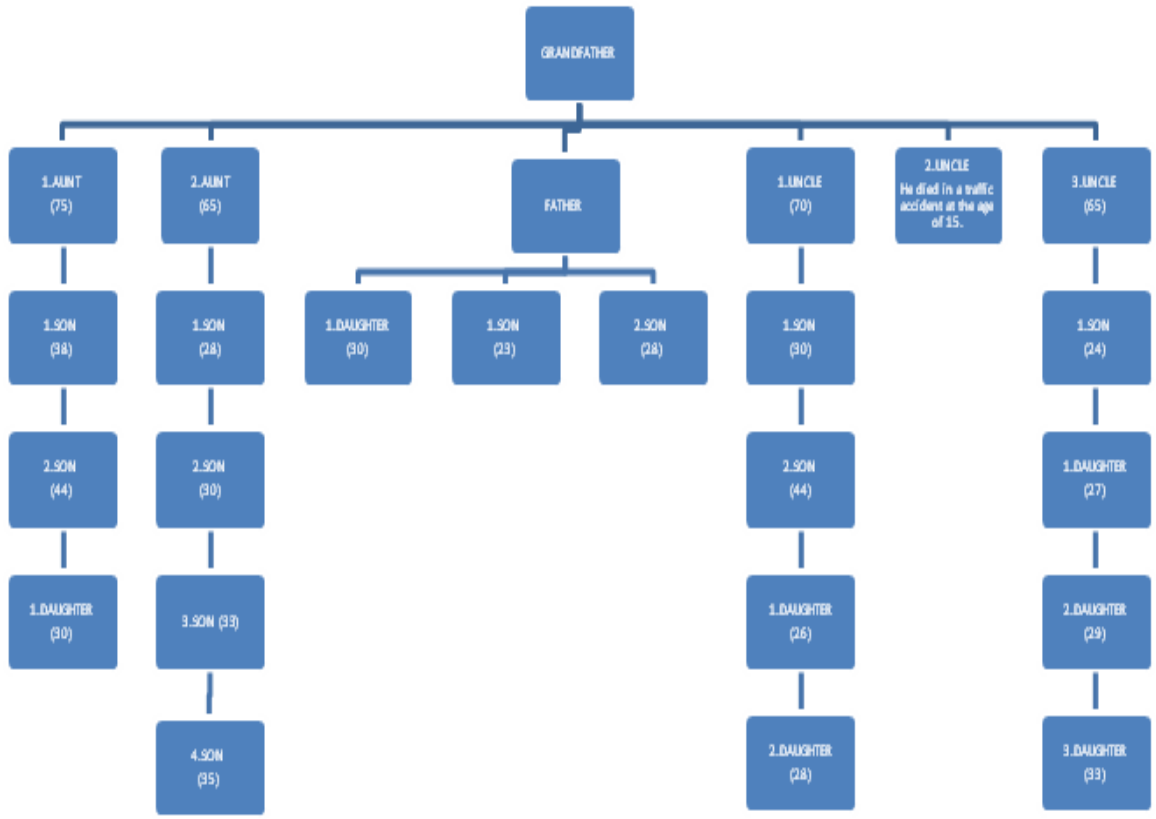
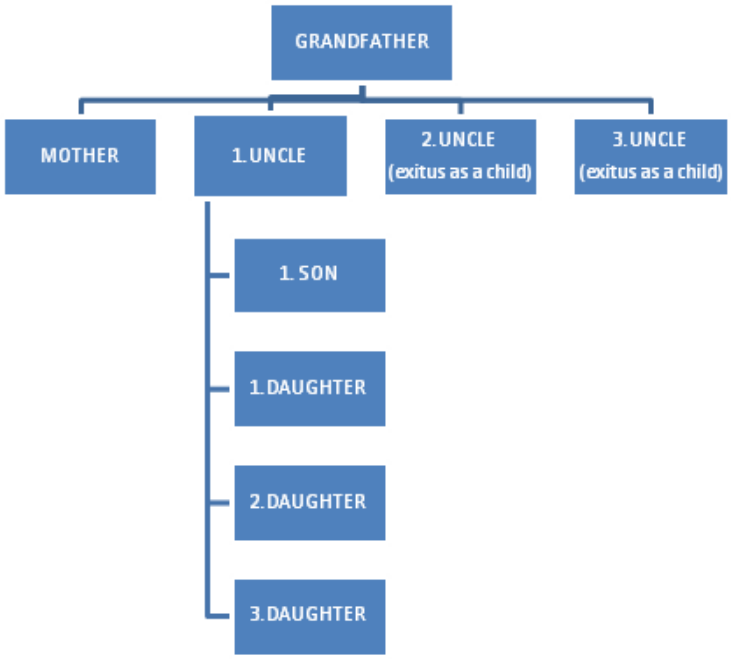


Figure2: *Genetic map of the patient*

Note: *One son and one daughter of third uncle had mental reterdation*



2.2. Genetic Analysis

Patient 1 was diagnosed with CPT2 deficiency, myopathy, stress-indruced (MIM NO: 255110) using next-generation sequencing (NGS) combined with Sanger sequencing for the definite diagnosis. We detected a homozygous missense mutation (c.338C>T/p.Ser113Leu) on the CPT2 (NM_000098) gene. The same mutation was detected homozygously in the brother of the patient with the sanger method (Figure 3).

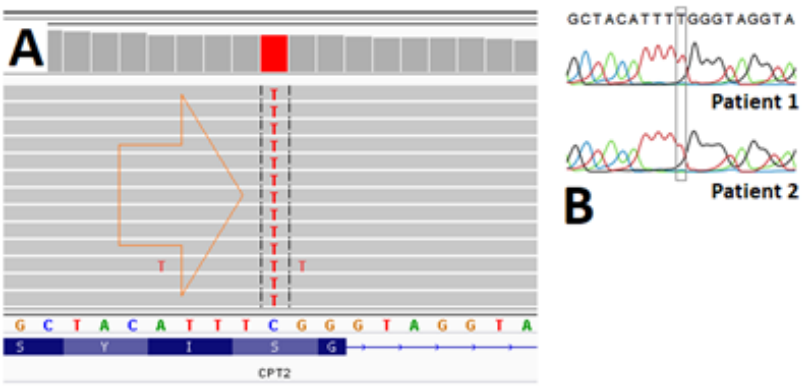


Figure3: *Genetic analysis*

2.3. Gene Detection Methods

In the present study, clinical exome sequencing and Sanger sequencing were performed, and genomic DNA was extracted from peripheral venous blood using the QIAamp DNA Mini Kit (Qiagen, Ankara, Turkey). The Clinical Exome Solution (Sophia Genetics SA, Saint-Sulpice, Switzerland) was used for exome enrichment, with all procedures carried out according to the manufacturer's protocols. This capture-based target enrichment kit covers 4493 genes with known inherited diseases causing mutations. Paired-end sequencing was performed on NextSeq 500 system (Illumina, San Diego, CA, USA) with a read length of 150_2, while the base calling and image analysis were conducted using Real-Time Analysis (integrated to the NextSeq 500 system; Illumina) software. The BCL (base calls) binary is converted into FASTQ utilizing the Illumina package bcl2fastq. All bioinformatics analyses were performed on Sophia DDM^{TM} platform (Sophia Genetics SA), which includes algorithms for alignment, calling single nucleotide polymorphisms (SNPs) and small insertions/deletions (Pepper^{TM}, Sophia Genetics SA patented algorithm) calling copy number variations (Muskat^{TM}, Sophia Genetics SA patented algorithm) and functional annotations (Moka^{TM}, Sophia Genetics SA patented algorithm). The raw reads were aligned to the human reference genome (GRCh37/hg19). Variant filtering and interpretations were performed on the Sophia DDM^{TM} platform (Sophia Genetics SA), and an Integrative Genomics Viewer (IGV) was used to visualize the BAM (binary alignment map) files (5).

3. DISCUSSION

A male patient who developed AKI as a result of rhabdomyolysis and diagnosed with kidney biopsy and myoglobin cylinder nephropathy, additionally his brother with a similar history presented in our case report. CPT2 deficiency diagnosed by genetic analysis for both patients. In case of rhabdomyolysis myoglobin levels can exceed the protein-binding capacity of the plasma, and myoglobin precipitates in the glomerular filtrate. The mechanical obstruction of tubules by myoglobin is an important factor causing AKI. Other contributing factors of AKI are vasoconstriction, hypovolemia, and direct renal toxic effect of myoglobin (6, 7).

There was no medical history of trauma, non-traumatic exertional (overexertion in untrained individuals, hyperthermia, or metabolic myopathies) or non-exertional (drugs or toxins, infections, alcohol, drug abuse or electrolyte disorders) in both cases. Both siblings presented with AKI with rhabdomyolysis and dialysis at the same ages and they healed only with supportive treatment. The diseases did not repeat after following years. However, the family had a history of unknown death and mental retardation. No genetic analysis was made to anyone except two brothers.

Hereditary causes are genetic disorders associated with terminal glycolysis or glycogenolysis, lipid metabolism, crebs cycle, mitochondrial respiratory chain, myopathies and others. A hereditary cause should be suspected in case of a positive family history for neuromuscular disorders, concomitant presence of exercise intolerance, recurrent muscle cramps and recurrent rhabdomyolysis. The metabolic myopathies represent a very small percentage of cases of rhabdomyolysis overall but are relatively common causes among patients with recurrent episodes of rhabdomyolysis after exertion. The biochemical consequences of the disease causing mutations of muscle carnitine palmitoyltransferase II (CPT II) deficiency are still enigmatic. Therefore, CPT II was

characterized in muscle biopsies of nine patients with genetically proven muscle CPT II deficiency. Total CPT activity (CPT I+CPT II) of patients was not significantly different from that of controls. Remaining activities upon inhibition by malonyl-CoA and Triton X-100 were significantly reduced in patients. Immunohistochemically CPT II protein was predominantly expressed in type-I-fibers with the same intensity in patients as in controls. Western blot showed the same CPT II staining intensity ratio in patients and controls. CPT I and CPT II protein concentrations estimated by ELISA were not significantly different in patients and in controls. Citrate synthase activity in patients was significantly increased. Total CPT activity significantly correlated with both CPT I and CPT II protein concentrations in patients and controls. This implies (i) that normal total CPT activity in patients with muscle CPT II deficiency is not due to compensatory increase of CPT I activity and that (ii) the mutant CPT II is enzymatically active. The data further support the notion that in muscle CPT II deficiency enzyme activity and protein content are not reduced, but rather abnormally inhibited when fatty acid metabolism is stressed. Carnitine acyltransferases catalyze the reversible transfer of acyl groups from acyl-coenzyme A esters to l-carnitine, forming acyl-carnitine esters that may be transported across cell membranes. l-Carnitine is a wáter-soluble compound that humans may obtain both by food ingestion and endogenous synthesis from trimethyl-lysine. Most l-carnitine is intracellular, being present predominantly in liver, skeletal muscle, heart and kidney. The organic cation transporter-2 facilitates l-carnitine uptake inside cells. Congenital dysfunction of this transporter causes primary l-carnitine deficiency. Carnitine acetyltransferase is involved in the export of excess acetyl groups from the mitochondria and in acetylation reactions that regulate gene transcription and enzyme activity. Carnitine octanoyltransferase is a peroxysomal enzyme required for the complete oxidation of very long-chain fatty acids and phytanic acid, a branched-chain fatty acid. Carnitine palmitoyltransferase-1 is a transmembrane protein located on the outer mitochondrial membrane where it catalyzes the conversion of acyl-coenzyme A esters to acyl-carnitine esters. Carnitine acyl-carnitine translocase transports acyl-carnitine esters across the inner mitochondrial membrane in exchange for free l-carnitine that exits the mitochondrial matrix. Carnitine palmitoyltransferase-2 is anchored on the matrix side of the inner mitochondrial membrane, where it converts acyl-carnitine esters back to acyl-coenzyme A esters, which may be used in metabolic pathways, such as mitochondrial β-oxidation. l-Carnitine enhances nonoxidative glucose disposal under euglycemic hyperinsulinemic conditions in both healthy individuals and patients with type 2 diabetes, suggesting that l-carnitine strengthens insulin effect on glycogen storage. The plasma level of acyl-carnitine esters, primarily acetyl-carnitine, increases during diabetic ketoacidosis, fasting, and physical activity, particularly high-intensity exercise. Plasma concentration of free l-carnitine decreases simultaneously under these conditions. CPT (carnitine palmitoyltransferase) II muscle deficiency is the most common form of muscle fatty acid metabolism disorders. In contrast to carnitine deficiency, it is clinically characterized by attacks of myalgia and rhabdomyolysis without persistent muscle weakness and lipid accumulation in muscle fibers. The biochemical consequences of the disease-causing mutations are still discussed controversially. CPT activity in muscles of patients with CPT II deficiency ranged from not detectable to reduced to normal. Based on the observation that in patients, total CPT is completely inhibited by malony-CoA, a deficiency of malonyl-CoA-insensitive CPT II has been suggested. In contrast, it has also been shown that in muscle CPT II deficiency, CPT II protein is present in normal concentrations with normal enzymatic activity. However, CPT II in patients is abnormally sensitive to inhibition by malonyl-CoA, Triton X-100 and fatty acid metabolites. A recent study on human recombinant CPT II enzymes (His_6-N-hCPT2 and His_6-N-hCPT2/S113L) revealed that the wild-type and the S113L variants showed the same enzymatic activity. However, the mutated enzyme showed an abnormal thermal destabilization at 40 and 45 °C and an abnormal sensitivity to inhibition by malony-CoA. The thermolability of the mutant enzyme might explain why symptoms in muscle CPT II deficiency mainly occur during prolonged exercise, infections and exposure to cold. In addition, the abnormally regulated enzyme might be mostly inhibited when the fatty acid metabolism is stressed. In muscle biopsies of 77 consecutive patients with idiopathic myoglobinuria, specific enzyme deficiencies were identified in 36 (47%). CPT deficiency

(47.2%, n=17) was the most common disorder, followed by muscle phosphorylase deficiency (McArdle disease, 27.7%, n=10), phosphorylase kinase deficiency (n=4), myoadenylate deaminase (MAD) deficiency (n=3), phosphoglycerate kinase deficiency (n=1) and a combined defect of CPT and MAD (n=1). Rhabdomyolysis may develop in patients with abnormal muscle, such as individuals with inherited disorders of glycogenolysis, glycolysis, or lipid metabolism (8-12). Deficiency of carnitine palmitoyltransferase type II (CPT2) is a disorder of lipid metabolism that, in the muscle form, manifests with recurrent attacks of myalgias often associated with myoglobinuria. Rhabdomyolytic episodes may be complicated by life-threatening events, including AKI. The carnitine cycle shuttles long-chain fatty acids from the cytosol into the mitochondrial matrix where fatty acid oxidation occurs. The presentations of carnitine cycle disorders vary with age; newborns and infants tend to present with more severe multisystemic disease triggered by infection or fasting, often with acute encephalopathy, liver failure, and cardiac involvement, while older children and adults tend to present with exercise-induced myalgias, weakness, and fatigue. A severe neonatal form of CPT2 deficiency presents with hypotonia, cardiomyopathy, arrhythmias, seizures, and multiple congenital anomalies (dysmorphic facies, renal cysts, brain malformations) and may result in death during the first days to months of life. However, the majority of affected individuals have a later-onset form that presents in the second or third decade of life with exercise intolerance and attacks of rhabdomyolysis, which can lead to renal failure and death (13-14).

Carnitine palmitoyltransferase 2 (CPT2) deficiency, the most common inherited disease of the mitochondrial long-chain fatty acid (LCFA) oxidation, may result in distinct clinical phenotypes, namely a mild adult muscular form and a severe hepatocardiomuscular disease with an onset in the neonatal period or in infancy. In order to understand the mechanisms underlying the difference in severity between these phenotypes, we analyzed a cohort of 20 CPT2-deficient patients being affected either with the infantile (seven patients) or the adult onset form of the disease (13 patients). Using a combination of direct sequencing and denaturing gradient gel electrophoresis, 13 CPT2 mutations were identified, including five novel ones, namely: 371G>A (R124Q), 437A>C (N146T), 481C>T (R161W), 983A>G (D328G), and 1823G>C (D608H). After updating the spectrum of CPT2 mutations (n=39) and genotypes (n=38) as well as their consequences on CPT2 activity and LCFA oxidation, it appears that both the type and location of CPT2 mutations and one or several additional genetic factors to be identified would modulate the LCFA flux and therefore the severity of the disease (15).

Finally, the history of patients, kidney biopsy and genetic analysis are important in terms of diagnosis and treatment in unknown causes of AKI. Two brothers were farmer in our case report. They weren't aware of doing strenuous exercise that could trigger rhabdomyolysis. As a result of genetic analysis in our cases, CPT II deficiency associated with lipid metabolism was diagnosed as the cause of rhabdomyolysis. All other etiological causes excluded clinical and laboratory, severe exercise, hunger or infection could cause this condition. In the following years, they did not have any similar complaints and their biochemical tests remained within normal limits.

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Citation: *Mehmet Usta, Alparslan Ersoy, Baris Done1, Yavuz Ayar, Işın Kılıçaslan, Ozlem Gorukmez, A Case of Myoglobin Cast Nephropathy with a Variant of Homozygote Mutation in Carnitinepalmitoy ltransferase2 (CPT2) Gene. ARC Journal of Nephrology, 2020; 5(1):21-27.*