Carnitine Palmitoyltransferase II Deficiency Due to a Novel Gene Variant in a Patient With Rhabdomyolysis and ARF

Hidetoshi Kaneoka, MD, PhD, Noriko Uesugi, MD, PhD, Ayaka Moriguchi, PharB, Shinichi Hirose, MD, PhD, Masaki Takayanagi, MD, PhD, Seiji Yamaguchi, MD, PhD, Yosuke Shigematsu, MD, PhD, Tetsuhiko Yasuno, MD, Yoshie Sasatomi, MD, PhD, and Takao Saito, MD, PhD

• **Adult patients deficient in carnitine palmitoyltransferase II (CPT II) cannot generate sufficient amounts of energy, which results in rhabdomyolysis and acute renal failure (ARF). Its genetic basis has been recognized; but histopathologic changes, especially electron microscopic changes, have scarcely been described. The study subject is a patient with ARF caused by repetitive nontraumatic rhabdomyolysis. The acylcarnitine profile of serum and enzyme assay on skin fibroblasts confirmed the diagnosis of CPT II deficiency. Renal biopsy specimens were examined microscopically and immunohistochemically. The histological diagnosis was interstitial nephritis with acute tubular necrosis caused by rhabdomyolysis. Myoglobin in tubules was detected by means of immunohistochemistry and electron microscopy. The genetic structure of CPT II was analyzed in the patient and his family. Eight pairs of polymerase chain reaction (PCR) primers were designed to cover the coding region. Each PCR-amplified gene product was subjected to DNA sequencing, which unveiled heterozygosity at the CPT II locus consisting of a deletion of cytosine and thymine at codon 408, resulting in a stop signal at 420, as well as a mutation of arginine to cysteine at codon 631. The frame shift at 408 has never been described before. DNA sequencing of the family showed the deletion mutation from the mother and the point mutation from the father. We describe renopathological findings in a patient with CPT II deficiency associated with rhabdomyolysis, which suggested the pathological role of myoglobin casts in the development of tubular necrosis. Genetic analysis of the patient identified a novel variant of the CPT II gene. *Am J Kidney Dis* 45:596-602.**

INDEX WORDS: Rhabdomyolysis; acute renal failure (ARF); carnitine palmitoyltransferase II (CPT II) deficiency; frame shift; point mutation; electron microscopy.

CARNITINE palmitoyltransferase II (CPT II) is one of the key mitochondrial enzymes involved in β-oxidation of long-chain fatty acids. β-oxidation is activated when there is an immediate requirement for high levels of energy, for example, in exhausting sports, military exercises, and severe infection. Adult patients deficient in this enzyme cannot supply adequate amounts of energy, which results in acute rhabdomyolysis and, if not appropriately managed, acute renal failure (ARF).[1-3] There is sufficient evidence for a genetic basis of this disorder; however, the associated histopathologic findings, especially electron microscopic findings, have rarely been described. We report a case of ARF caused by repetitive and nontraumatic rhabdomyolysis resulting from CPT II deficiency. Renal biopsy specimens were examined by means of light microscopy, electron microscopy, and immunohistochemistry. Genetic structure and familial segregation also were determined.

CASE REPORT

A 24-year-old Japanese man was transferred to the emergency department of Fukuoka University Hospital and Clinic (Fukuoka, Japan) because of ARF. Three years before presentation, he developed an illness characterized by high fever, myalgia, respiratory symptoms, and dark-colored urine. After hospitalization, he was found to have markedly elevated

From the Departments of Internal Medicine, Pathology, and Pediatrics, Division of Nephrology and Rheumatology, Fukuoka University School of Medicine, Fukuoka; Chiba Children's Hospital, Chiba; Department of Pediatrics, Shimane University, Izumo, Japan; and Department of Health Science, University of Fukui, Fukui, Japan.

Received August 9, 2004; accepted in revised form December 1, 2004.

Originally published online as doi:10.1053/j.ajkd.2004.12.006 on January 27, 2005.

Supported in part by fund no. 026006 from the Central Research Institute of Fukuoka University and a grant for Progressive Renal Diseases from the Ministry of Health, Labor, and Welfare, Japan.

Presented in abstract form at the American Society of Nephrology Annual Meeting, St Louis, MO, October 31, 2004.

Address reprint requests to Hidetoshi Kaneoka, MD, PhD, Division of Nephrology and Rheumatology, Department of Internal Medicine, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-Ku, Fukuoka 814-0180, Japan. E-mail: kaneokah@fukuoka-u.ac.jp

0272-6386/05/4503-0018$30.00/0

doi:10.1053/j.ajkd.2004.12.006

Table 1. CPT I and II Activity in Cultured Skin Fibroblasts

	CPT I Activity		
	Without Malonyl Coenzyme A	With Malonyl Coenzyme A	CPT II Activity
Present case	0.90	0.20	0.02
CPT I deficiency	0.01	0.08	0.80
Healthy controls (n = 10)*	1.38 ± 0.54	0.30 ± 0.11	0.97 ± 0.25

NOTE. CPT activity expressed as nanomoles of palmitoyl-L-(methyl-^{14}C) carnitine per minute per milligrams of protein. CPT I activity is expressed as the difference in CPT I activity in the presence and absence of 50 μmol/L of malonyl-coenzyme A, which is a specific inhibitor of CPT I. CPT I and II activity was measured in cultured skin fibroblasts as described by Demaugre et al.[5] Briefly, activities were assayed in supernatant of fibroblast homogenates as palmitoyl-L-(methyl-^{14}C) carnitine formed from L-(methyl-^{14}C) carnitine and palmitoyl coenzyme A. Palmitoyl-L-(methyl-^{14}C) carnitine was extracted with isopropanol and measured for CPT I activity.

*Data expressed as mean ± SD.

levels of serum muscle enzymes and urine myoglobin, and a diagnosis of rhabdomyolysis, probably caused by a viral infection or drugs prescribed for a common cold, was made. He was treated by means of assisted ventilation through endotracheal intubation, hydration, and medical diuresis and discharged 2 weeks later without complications. During that admission, renal dysfunction was not recognized.

In the current illness, the patient reported high fever, general malaise, myalgia, flulike symptoms, dyspnea, and dark-colored urine. He was seen by the family physician and referred to a consultant at a local medical center, where he was found to have rhabdomyolysis and ARF, and he was transferred to our unit. He was born to a nonconsanguinous couple. He smoked 40 cigarettes per day and was an occasional drinker. Physical examination on admission was not remarkable, with alert consciousness, normal neurological signs and reflexes, and arterial blood pressure of 150/96 mm Hg. Initial laboratory investigations showed positive test results for inflammation (circulating leukocytes, 17.4 × $10^3/\mu$L [× 10^9/L]; C-reactive protein, 21.5 mg/dL), high serum levels of myogenic enzymes (aspartate aminotransferase, 1,645 IU/L; alanine aminotransferase, 571 IU/L; lactic dehydrogenase, 4,340 IU/L; creatine kinase, 127,600 IU/L; aldolase, 132.8 U/L [normal, 0.5 to 3.1 U/L]; and myoglobin, 63,000 ng/mL [normal, <60]), and evidence of renal damage (proteinuria, 2^+ by means of test strips; myoglobinuria with myoglobin of 370,000 ng/mL; blood urea nitrogen, 27 mg/dL [9.6 mmol/L]; serum creatinine, 3.1 mg/dL [274 μmol/L]; β_2-microglobulin, 21.5 mg/L [normal, 0.8 to 1.8 mg/L]; and 24-hour creatinine clearance, 15 mL/min [0.25 mL/s]). Urine sediments showed 1 to 4 red blood cells and 10 to 20 white blood cells per high-power field, as well as a few granular and epithelial casts per low-power field. Serological test results for toxoplasma, mycoplasma, and antinuclear autoantibodies were negative. Lymphocyte stimulation tests for drugs administered for the flulike symptoms were negative. Diagnoses of rhabdomyolysis and ARF were made. The patient recovered successfully from ARF with hydration and diuresis and continuous hemofiltration and hemodialysis for 4 consecutive days, with resultant normalization of 24-hour urine volumes.

The repetitive rhabdomyolysis and complete recovery from ARF prompted us to investigate genetic and metabolic disorders in this patient after obtaining a signed consent form. Skin fibroblasts were surgically harvested from the forearm, cultured, and used for additional metabolic and genetic analyses. The acylcarnitine profile in a serum sample obtained during the acute phase by using tandem mass spectrometry showed markedly elevated levels of long-chain acylcarnitines, suggesting the diagnosis of CPT II deficiency (data not shown).[4] Enzyme assay using cultured skin fibroblasts showed low enzymatic activity and confirmed the diagnosis of CPT II deficiency[5] (Table 1).

Immunohistochemical and Electron Microscopy

One month after the onset, serum creatinine and 24-hour creatinine clearance values were 1.4 mg/dL (124 μmol/L) and 27 mL/min (0.45 mL/s), respectively. Urinalysis still showed 1^+ proteinuria with a few granular and epithelial casts. To determine the significance and extent of renal involvement, rather than its existence, a renal biopsy was performed. The obtained tissue sample was examined by means of light microscopy, immunohistochemistry, and electron microscopy, using methods described previously.[6] For light microscopy, paraffin-embedded sections were stained with hematoxylin-eosin, periodic acid–Schiff, periodic acid–methenamine silver, and Masson trichrome. For immunohistochemistry, sections were stained with polyclonal antisera to human immunoglobulin G (IgG), IgA, IgM, complements 1q and 3, and fibrinogen. Immunoperoxidase staining also was performed using rabbit antihuman myoglobin antibody (Dako, Glostrup, Denmark).

Light microscopic examination showed no remarkable changes in glomeruli; however, patchy and dense cellular infiltration was identified in the interstitium, together with acute necrosis of the proximal tubules. Distal tubules contained coarse granular light-brown pigmented casts (Fig 1A) and other protein-rich casts. Necrosis of tubular epithelia, disruption of the tubular basement membrane, and inflammatory cells were seen in several distal tubules, especially those containing pigmented casts. Dense inflammatory infiltrates, composed mainly of lymphocytes, histiocytes, neutrophils,