SHORT REPORT

ABSTRACT: The most common mutation in muscle carnitine palmitoyltransferase II (CPT II) deficiency is a missense mutation that replaces a leucine for a serine residue at amino acid position 113 of the CPT II protein (S113L). We performed molecular analysis in a group of 14 Spanish patients with CPT II deficiency from ten unrelated families. The S113L mutation was observed in 8 of the 14 patients studied. Seven patients were homozygous for the mutation, 1 patient was heterozygous, and 6 patients did not carry the mutation on either allele. Seven healthy relatives belonging to three different families carried the mutation on one allele. One patient carried the missense mutation that replaces a tyrosine for a serine at amino acid position 628 on one allele. Our data indicate that the S113L is also the most common mutation in Spanish patients with CPT II deficiency in muscle, and that further pathogenic mutations remain to be identified.

 Muscle Nerve **22:** 941–943, 1999

MOLECULAR ANALYSIS IN SPANISH PATIENTS WITH MUSCLE CARNITINE PALMITOYLTRANSFERASE DEFICIENCY

MIGUEL A. MARTÍN, Pharm B, JUAN C. RUBIO, MSc, FERNANDO DE BUSTOS, MSc, PILAR DEL HOYO, Pharm B, YOLANDA CAMPOS, MSc, ALBERTO GARCÍA, MSc, BELÉN BÖRNSTEIN, MD, ANA CABELLO, MD, and JOAQUÍN ARENAS, PhD

Centro de Investigación, Hospital Universitario 12 de Octubre, Madrid, Spain

Accepted 26 February 1999

The carnitine palmitoyltransferase (CPT) enzyme system consists of two distinct mitochondrial membrane-bound enzymes: CPT I, located on the inner side of the outer membrane; and CPT II, situated on the inner membrane.[7] Whereas CPT I exists as tissue-specific isoforms, CPT II does not.[7]

CPT II deficiency is the most common recessively inherited disorder of lipid metabolism affecting skeletal muscle. Typically, it presents in young adults with recurrent episodes of exercise-induced myoglobinuria.[3,16] Atypical phenotypes of CPT II deficiency include hypoketotic hypoglycemia, cardiomyopathy and sudden death in newborns and children,[1,5] recurrent pancreatitis,[12] and brain and kidney dysplasia.[8]

The CPT II gene has been cloned, and assigned to chromosome 1p32.[4] Several CPT II mutations have been detected, but the most common mutation in adult CPT II deficiency is a missense mutation that replaces a leucine for a serine residue at amino acid position 113 of the CPT II protein (S113L).[10] An arginine-to-cysteine substitution at amino acid residue 631 (R631C) was initially described in an infant with hypoketotic hypoglycemia and cardiomyopathy and was also found in heterozygous form in 2 patients with the adult phenotype of CPT II deficiency.[11] A proline-to-histidine substitution at amino acid position 50 (P50H) was found in 4 patients, 1 of them being homozygous for the mutation and the other 3 heterozygous.[13] Other, more rarely occurring mutations have also been reported.[13–15] In infantile and neonatal CPT II deficiency, a tyrosine-to-serine missense mutation at amino acid position 628 (Y628S) has also been described.[1]

We describe here molecular genetics findings in 14 Spanish patients with muscle CPT II deficiency.

Abbreviations: CK, creatine kinase; CPT, carnitine palmitoyltransferase; nt, nucleotide

Key words: molecular analysis; carnitine palmitoyltransferase II; myoglobinuria; exercise intolerance; muscle

Correspondence to: Joaquín Arenas

CCC 0148-639X/99/070941-03

METHODS

Our group of patients with CPT II deficiency consisted of 14 patients from ten unrelated families. Patients 2 and 3 were siblings, as also were patients 8

and 9. Twelve patients had the typical clinical phenotype of muscle CPT II deficiency with recurrent episodes of myoglobinuria, triggered by prolonged exercise, fasting, or fever. Serum creatine kinase (CK) values were dramatically elevated during metabolic crisis. Acute renal failure occurred only in patient 1. Patients 3 and 13 had exercise-related myalgia, cramps, and moderate elevation of serum CK values, but had never had myoglobinuria. The main clinical findings are summarized in Table 1.

Muscle morphology showed lipid storage myopathy in patient 2, and was normal in the remaining patients. The activity of CPT II in muscle was measured as described previously,[9] but with slight modifications, namely the use of 0.3 mmol/L of palmitoylcarnitine. Normal values were 0.22–0.60 nmol/min · mg noncollagen protein (mean value 0.42). Residual CPT II activities in muscle ranged from 1% to 48% of mean control value (Table 1).

We studied DNA from muscle biopsy specimen in 13 patients with biochemically proven CPT II deficiency. In patient 3, who was the sibling of patient 2, as muscle biopsy and biochemical studies were not performed, DNA from peripheral leukocytes was studied. We also examined blood DNA from 2 healthy relatives of patients 2 and 3, from 5 healthy relatives of patient 5, and from 3 healthy relatives of patient 14. Genomic DNA was extracted according to standard procedures. We looked for four missense mutations (S113L, R631C, P50H, and Y628S) by PCR amplification and restriction enzyme digestion using primer sets and protocols as previously described.[1,10,11,13] In the R631C substitution, the underlying C-to-T transition at nucleotide (nt) 2407 creates a *Bbv* I site. The C-to-T transition at nt 854 (S113L) and the C-to-A transversion at nt 665 (P50H) do not result in a restriction site, so that mismatches have to be incorporated into the primer to generate a restriction site in the mutant sequence. The amplified fragment was then digested with *BstX* I and *Dra* III, respectively. In the Y628S substitution, the A-to-C transversion at nt 2399 creates a *Mnl* I site. Fragments were visualized following electrophoresis on ethidium-bromide-stained agarose gel. In particular, in the presence of the S113L mutation, the 167-nt fragment is cut into two smaller fragments of 137 and 30 nt, whereas in its absence the 167-nt fragment remains uncut.

RESULTS

The R631C and P50H missense mutations were not found in our patients (Table 1). The S113L mutation was observed in 8 of the 14 patients studied. Seven patients were homozygous for the mutation, 1 patient was heterozygous, and 6 patients did not carry the mutation on either allele (Table 1). In patient 3, the presence of the mutation was established by analysis of DNA from blood cells. The following relatives studied were carriers of the S113L mutation: the father and the mother of patients 2 and 3; the father, the mother, two sisters, and the daughter of patient 5; and the mother and one brother of patient 14. The eldest brother of patient 14 did not carry the mutation. Patient 12 was heterozygous for the Y628S mutation.

DISCUSSION

CPT II deficiency is one of the most common recessively inherited disorders of lipid metabolism. It presents in young adults with recurrent episodes of myoglobinuria induced by prolonged exercise, fever, and fasting, sometimes leading to acute or chronic renal failure.[3,16] The majority of our patients showed this typical clinical pattern. Patients 3 and 13, however, had a less severe phenotype, in accord with the absence of myoglobinuria and moderate elevation of serum CK. Recurrent episodes of myoglobinuria with marked elevation of serum CK values were present in 86% of the patients, leading to renal failure

Table 1. Clinical, biochemical, and molecular findings in patients with CPT II deficiency in muscle.

	Patient no.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gender	M	M	F	F	F	F	M	M	M	M	M	M	M	M
Age at onset	14	8	14	24	17	12	10	12	14	16	27	6	7	14
Age at biopsy	39	10	18	41	32	23	18	21	17	19	28	21	11	21
Myoglobinuria	+	+	−	+	+	+	+	+	+	+	+	+	−	+
Muscle CPT residual activity (nmol/min · mg NCP)	0.15	0.03	ND	0.08	0.20	0.06	0.02	0.04	0.10	0.05	0.06	0.004	0.03	0.11
Molecular analysis														
S113L	–/–	+/+	+/+	–/–	+/+	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/–	+/–
P50H	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
R631C	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
Y628S	–/–	–/–	–/–	–/–	ND	–/–	–/–	–/–	ND	ND	–/–	+/–	–/–	–/–

Mean normal value for CPT = 0.42 nmol/min · mg NCP. M, male; F, female; ND, not done; NCP, noncollagen protein.

solely in patient 1. Muscle morphology was unremarkable, except for the presence of lipids in patient 2.

Consistent with previous reports,[2,16] we observed a wide range of residual CPT II activities in muscle. Demaugre et al.[2] hypothesized that the magnitude of residual CPT II activity determines the severity and tissue specificity of the symptoms. However, our data do not seem to support this hypothesis. For instance, patient 1 showed a severe phenotype with acute renal failure despite having as much as 35% residual CPT II activity in muscle, whereas patient 13 showed a milder phenotype with no pigmenturia, although he had only 7% residual muscle CPT II activity. Moreover, two siblings (patients 8 and 9), with a similar severe phenotype, showed substantial differences in residual CPT II activities in muscle.

The most common mutation in adult CPT II deficiency is the S113L substitution. Taroni et al.[10] found this mutation in 80% of a group of 25 Italian patients, of whom 8 were homozygous for the mutation and 12 heterozygous. Zierz et al.[17] reported that 86% of their 22 German patients harbored the mutation, of whom 8 were homozygous and 12 heterozygous. More recently, Kauffman et al [6] observed the mutation in 95% of a group of 20 North American patients, of whom 5 proved to be homozygous and 14 heterozygous. In our group, only 8 of 14 (57%) patients carried the mutation. Although the proportion of mutant alleles (15 of 28) was comparable to that found in previous studies,[6,10,17] their distribution proved to be remarkably different; that is, although 7 patients were homozygous for the S113L mutation, only 1 was heterozygous. This amounts to 50% homozygosity, compared with 25–36% in previous reports.[6,10,17]

Interestingly, patient 12, who had the earliest onset of symptoms, carried the Y628S missense mutation on one allele. This mutation has been shown to be associated with infantile and neonatal CPT II deficiency,[1] but has never been found in patients with the typical muscle CPT II deficiency phenotype. In addition, heterozygosity for the R631C mutation has been observed in 2 patients with the adult phenotype of CPT II deficiency.[11]

Our data indicate that the S113L is also the most common mutation in Spanish patients with CPT II deficiency in muscle, and that there are further pathogenic mutations that remain to be identified. Moreover, in patients with myoglobinuria of unknown origin, specific PCR screening for the S113L mutation may be helpful for diagnosis.

Supported by grants from Fondo de Investigaciones Sanitarias (FIS) (nos. 98/1413 and 98/258), Ministerio de Sanidad. Spain. M.A. Martín was supported by a grant from FIS (no. 98/1413), J.C. Rubio by a grant from the Instituto de Salud Carlos III (ISC III) (no. 97/460), and Y. Campos by a grant from Sigma-Tau

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