SHORT REPORT

ABSTRACT: Carnitine palmitoyltransferase II (CPT II) deficiency is the most common lipid myopathy in adults and is characterized by exercise-induced pain, stiffness, and myoglobinuria. Retrospective analysis of patients with CPT II deficiency has made it possible to correlate the presence of disease-causing mutations in the *CPT2* gene with residual CPT activity in muscle. We present evidence that the ratio of CPT II activity to citrate synthase activity in the skeletal muscle of patients presumed to have CPT II deficiency is important for predicting whether the patient has one, two, or no mutations in the *CPT2* gene. This finding will assist in the future correlation of the phenotype with the genotype and in identifying manifesting heterozygotes.

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BIOCHEMICAL AND MOLECULAR CORRELATIONS IN CARNITINE PALMITOYLTRANSFERASE II DEFICIENCY

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Carnitine palmitoyltransferase (EC 2.3.1.21; CPT) exists as two genetically and catalytically distinct mitochondrial enzymes (CPT I and CPT II).[1,3,9] Together with carnitine acylcarnitine translocase (CACT), they facilitate the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation.[8] Inherited defects in the *CPT2* gene cause several disorders including a rare lethal infantile disease that affects multiple organ systems (MIM #600649), a severe infantile disease with hepatocardiomuscular symptoms, and an adult-onset myopathic disease (MIM #255110).[13]

Until recently, the diagnosis of adult-onset CPT II deficiency required a muscle biopsy for enzymatic analysis. Since the human *CPT2* gene was first cloned and sequenced in 1991,[2] more than 15 disease-causing mutations of this gene have been reported.[12] Although mutation analysis is helpful and at least one mutation (S113L) accounts for 60% of the mutant alleles, and is identified in the majority of affected individuals,[14] mutation analysis is not completely diagnostic in every case. The identification of individual mutant alleles in patients has ranged from 60% to 77% in recent studies.[12] The advantage of mutation screening is that it may be performed as a relatively noninvasive initial screening of peripheral blood leukocytes[6] or buccal cells,[15] obviating the need for muscle biopsy.

We and others have identified symptomatic individuals who appear to be heterozygous for only one mutation.[6,12,14,15,18] Some of these individuals may have a second, as yet undetermined, mutation, whereas others may be manifesting carriers.[12] There have also been several reports of vertical transmission and partial CPT enzyme deficiencies, suggesting the existence of manifesting carriers.[4,5,7,15] Rigorous sequence analysis of the *CPT2* gene in several heterozygotes found in our study did not yield a second mutation in the five exons of the coding region and bordering intronic sequences. Furthermore, the intermediate concentration of residual activity in these patients, between that of known homozygotes and normal individuals, supports the possibility that manifesting carriers exist.[12]

We performed a retrospective study to determine the relationship between residual CPT enzyme activity in muscle and the number of *CPT2* mutations

Abbreviations: ASO, allele-specific oligonucleotide; CACT, carnitine acylcarnitine translocase; CPT II, carnitine palmitoyltransferase II; CS, citrate synthase; MIM, Mendelian Inheritance of Man

Key words: lipid myopathy; heterozygotes; disease-causing mutations; myoglobinuria; exercise intolerance

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present among patients with CPT II deficiency, thus creating a basis for the prediction of mutation status in patients referred for suspected CPT deficiency.

METHODS

CPT analysis was performed in whole homogenates of skeletal muscle biopsies using the radioisotope exchange method of Norum.[10] This method has been used successfully to detect homozygotes and heterozygotes for disease-causing mutations in *CPT2* with residual CPT activity ranging from 8% to 18% of the reference mean in skeletal muscle[12,17] and to detect comparable reductions of CPT activity in lymphoblasts.[16] Citrate synthase (CS), a marker for mitochondrial content, was quantified by the method of Srere.[11] *CPT2* mutation analysis and mutation scanning was performed using allele-specific oligonucleotides and dideoxynucleotide sequence analysis, respectively, as previously described.[12]

RESULTS AND DISCUSSION

The relationship between CPT activity, CS, and the number of mutations found in individuals with symptoms of CPT deficiency are presented in Table 1. Six individuals were homozygotes or compound heterozygotes for known mutations in *CPT2*. The ratio of CPT to CS in individuals with two mutations ranged from 0.47 to 1.00. There appeared to be no association between the amount of residual activity and the three different combinations of mutations present in these patients. However, the two patients who were homozygous for the S113L mutation had the lowest ratios among patents with two mutations in *CPT2*.

Three symptomatic individuals had only one disease-causing mutation detected by allele-specific oligonucleotide (ASO) screening for 15 mutations and mutation scanning by dideoxy sequence analysis.[12] Patient #1607 had a general reduction of mitochondrial respiratory chain enzymes in muscle to approximately 33% of normal, whereas nonmitochondrial enzyme activities remained within reference range, suggesting the presence of an underlying mitochondrial defect in addition to the carrier status for a *CPT2* mutation.[15] Based on residual CPT activity alone, this patient would have been suspected of being a homozygote or compound heterozygote for a *CPT2* mutation. Biochemical evidence for heterozygote status was only revealed when the residual CPT activity was expressed in a ratio with CS. Patient #4158, on the other hand, had the highest residual CPT activity in muscle among the homozygotes or compound heterozygotes for *CPT2* mutations. This patient's CS was 194% of normal, suggesting increased mitochondrial content in the muscle, thus accounting for the somewhat higher residual CPT activity found. The ratio of CPT to CS for this patient was the lowest ratio among individuals with two mutations.

Mutations could not be detected by ASO screening in 10 additional symptomatic individuals. Nine had CPT:CS ratios in the heterozygote range, whereas 1 individual had a ratio of 0.83, suggesting

Table 1. Correlation between CPT activity and *CPT2* mutations.

Patient no./group	CPT activity*	CS activity†	CPT:CS‡	*CPT2* mutations(s) detected
Simple heterozygotes (*n* = 3)				
1004	35.30 (45)	13.10	2.69	S113L/+
1003	30.12 (39)	17.54	1.72	S113L/+
1607	9.90 (13)	5.80	1.71	R503C/+
Mean ± SD	25.10 ± 10.96	12.15 ± 4.84	2.04 ± 0.46	
Presumed heterozygotes (*n* = 9)	26.21 ± 9.35	13.13 ± 3.40	1.97 ± 0.31	None
Homozygotes or compound heterozygotes (*n* = 6)				
1000	8.10 (10)	13.65	0.59	S113L/413 delAG-F448L
1002	13.80 (18)	13.77	1.00	S113L/413 delAG-F448L
3001	9.10 (12)	13.04	0.70	S113L/413 delAG-F448L
1614	10.12 (13)	17.16	0.59	P50H/G549D
3361	5.96 (8)	12.42	0.48	S113L/S113L
4158	14.37 (18)	30.48	0.47	S113L/S113L
Mean ± SD	10.24 ± 2.99	16.75 ± 6.32	0.64 ± 0.18	
Presumed homozygote or compound heterozygote (*n* = 1)	13.53	16.34	0.83	None
Myopathy patients without CPT deficiency (*n* = 44)	76.21 ± 7.11	16.42 ± 5.37	5.13 ± 1.62	

+, Normal allele; SD, standard deviation.
**Carnitine palmitoyltransferase reference mean activity ± SD: 77.80 ± 13.30 nmol/min/g tissue.[12]*
†Citrate synthase reference mean activity: 15.74 ± 4.40 μmol/min/g tissue.[12]
‡Expected reference ratio: 4.94.

that he may be a homozygote or compound heterozygote for two as yet undefined mutations in *CPT2*.

This study has shown that the most accurate identification of both homozygotes and heterozygotes for CPT II deficiency, as well as the distinction between the two categories, was made when CPT activity in muscle was expressed as a ratio with CS activity. This finding is especially important in patients with coexisting mitochondrial disorders. As seen with patients #1607 and #4158, the CPT:CS ratio reduces the potentially confounding effect of secondary mitochondrial abnormalities on the residual CPT activity. Therefore, unless this ratio is used for data expression, it is possible that patients may be misdiagnosed if their residual CPT activity is relatively high or low due to coexisting mitochondrial abnormalities. As biochemical and molecular evidence grows for the existence of manifesting carriers for certain mutations in *CPT2,* it is important to maximize the use of biochemical parameters to distinguish heterozygotes from compound heterozygotes and homozygotes when mutation analysis is of limited diagnostic value.

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