Carnitine palmitoyltransferase II deficiency

Molecular and biochemical analysis of 32 patients

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Abstract—The authors investigated 32 patients with the muscle form of CPT II deficiency. Total carnitine palmitoyltransferase enzyme system (CPT) activity was normal but abnormally inhibited by malonyl-CoA, palmitoyl-CoA, and the detergents Triton X and Tween 20. Mutation analysis identified three described mutations (S113L, P50H, and F448L) and two novel mutations (M214T and Y479F). Using modeling techniques, a structure could be identified anchoring the protein in the membrane. Only one of the five mutations (Y479F) is located within this region.

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The carnitine palmitoyltransferase enzyme system (CPT), consisting of CPT I and CPT II, mediates the entry of long-chain fatty acids into the mitochondrial matrix for β-oxidation.[1]

Adult-onset CPT II deficiency, characterized by exercise-induced muscle pain and weakness, rhabdomyolysis, and paroxysmal myoglobinuria, is the most common disorder of lipid metabolism affecting the skeletal muscle.[2] The human CPT II gene is located at chromosome 1, spans 20 kb, and contains five exons. A common mutation, S113L, is present in approximately 60% of alleles in addition to a multitude of rare, obviously private mutations.[3] There are conflicting reports on the biochemical consequences of CPT II deficiency caused by these mutations.[4,5]

Patients and methods. Thirty-two patients with CPT II deficiency and two asymptomatic parents were investigated. Control muscle specimens were obtained from patients who had muscle biopsies for diagnosis of muscular symptoms. Patients were deemed to be "normal controls" if they were ultimately found to have no muscle disease according to combined clinical, electromyographic, biochemical, and histologic criteria. Informed consent was obtained from all patients.

Total CPT activity was determined using the isotope forward assay as described.[4] The physiologic inhibitors malonyl-CoA and palmitoylcarnitine were added to the reaction mixture as indicated. In some experiments, membranes were disrupted by preincubation with Triton X-100 or Tween 20.

Genomic DNA was prepared from muscle tissue or blood using standard procedures. Polymorphisms and the S113L mutation were screened as published.[5] Primer pairs were synthesized to amplify the entire coding region and completely sequenced. Both strands of genomic DNA were analyzed to confirm a mutation. Restriction enzyme digestion-based assays were established to screen control subjects. Protocols have been published previously.[6]

Homology searching and alignment of the CPT II sequence was done with the FastA alogarithm.[8] Using the PHD neural network method of Rost and Sander, secondary structure and surface accessibility prediction was performed. The surface polarity of predicted helices was checked with an automated helical wheel projection procedure. Three-dimensional modeling of the α-helix bundle was done manually with the INSIGHT II software package (Molecular Simulations Inc., San Diego, CA).

Results. In all patients, CPT activity using the isotope forward assay was normal. Addition of the inhibitors malonyl-CoA and palmitoylcarnitine or the detergents Triton X and Tween decreased CPT activity in control subjects and patients, but the inhibition was significantly greater in patients than in control subjects (see table E- 1 on the Neurology Web site).

Fourteen index patients were homozygous and 17 were heterozygous for common the S113L mutation. One was homozygous for the P50H mutation. Two other patients were compound heterozygous for the S113L mutation and the P50H mutation. A T-to-C transition in nucleotide position 742 (nucleotide position according to Finocchiaro[3]) was found once (M214T); an A-to-T transition in position 1547 could be identified leading to the substitution of a tyrosine to a phenylalanine at position 479 (Y479F); and a T-to-C transition in position 1443 leading to a substitution of phenylalanine to leucine at position 448 (F448L) was found in three unrelated patients. This mutation was associated with a 2-base pair (bp) deletion at position 1339 and 1340 (413delAG). Fifty-five healthy control subjects did not have one of the described mutations. All results are summarized in table E-1.

A sequence database search found no other proteins homologous to CPT II except for other carnitine C-acyl transferases. However, when the homology search was repeated on short domains, a region was found that shares 50% identical residues and 75% similarity to a membrane-located helix of a mitochondrial ATPase. The domain F448-G497, comprising helix D464-Y479 and a second predicted helix (S488-H496) along with a region N-terminal to D464-Y479, is highly conserved among carnitine acyltransferases. For both helices, a low solvent accessibility is

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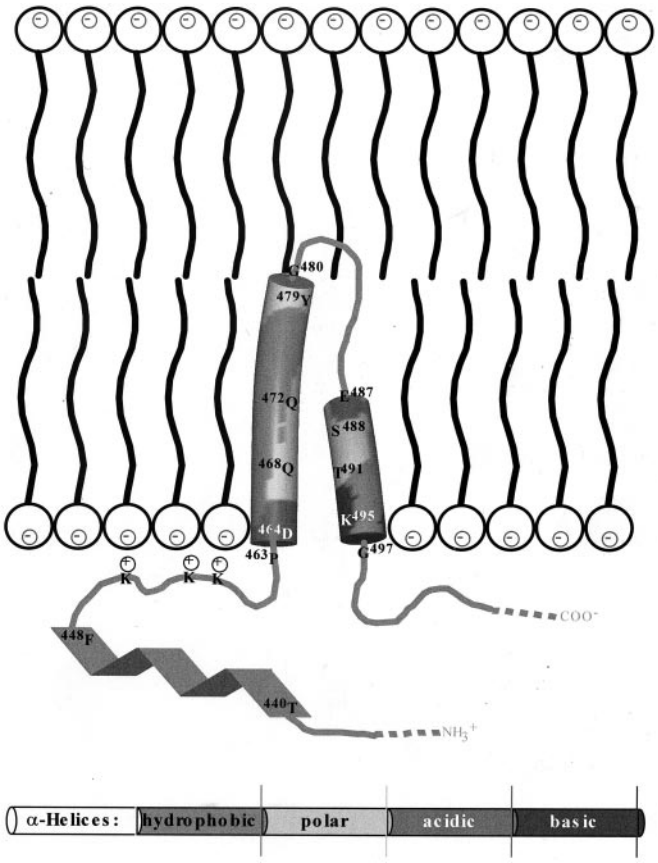

Figure 1. Model of the putative membrane anchor region of CPT II comprising residues T440-G497. The predicted helices D464-Y479 and S488-H496 are packed antiparallel facing each other with polar patches on one side of their surfaces. Additional salt bridges stabilizing the insertion of the helix-turn-helix structure in the lipid layer could be formed between negatively charged phospholipid head groups and a cluster of basic lysine residues located N-terminal to helix D464-Y479.

predicted, i.e., they are probably either buried inside the protein core or located in a lipid membrane. Based on conservation pattern, sequence characteristics, and predicted secondary structure, we propose that this region forms a membrane-associated domain with two predicted helices inserted into one layer of the lipid membrane. Helix D464-Y479 contains 16 residues, equivalent to a length of 2.4 nm, allowing it to traverse the unpolar hydrocarbon part of one layer of a lipid membrane. Thus, we propose a helix-turn-helix arrangement with parallel packing of the helices D464-Y479 and S488-H496 inside the membrane. A three-dimensional working model was constructed (figure 1).

Discussion. Consistent with previous data, the frequency of the S113L mutation in our sample was 73% in 56 unrelated alleles.[5] Of the other known mutations, we only found the P50H mutation in three patients. The M214T and Y479F mutations were detected only once and seem to represent private mutations. The localization of these mutations is shown in figure 2. Multiple sequence alignment of highly conserved domains of acyltransferases from different species revealed that the mutated amino acids in our patients are all highly conserved, indicating functional importance (table 1).

After our report of the F448L mutation in an abstract,[9] an association of the F448L mutation with a 2-bp deletion at amino acid position 413 in four patients was found.[7] Re-evaluation of our gels confirmed this association was present in our patients. The deletion results in a termination codon six amino acids downstream, which would leave the 448 mutation without functional importance. However, it is well known that the efficiency of any codon (stop codon in this case) is heavily influenced by its context. Therefore, the mode of action of this complex mutant haplotype is still enigmatic, and it remains to be proven which of the alterations is of functional relevance.

There was no obvious correlation of clinical characteristics, such as age at onset and frequency or severity of attacks, with any genotype (data not shown). Although biochemical properties seemed to be correlated with certain genotypes (e.g., Y479F is associated with high residual activity in the presence of Triton X-100), the small patient sample does not permit statistical analysis.

All genotypes found in our patients are restricted to the muscle form of CPT II deficiency and have not been found in the multisystemic form so far. Interestingly, when the 413delAG-F448L mutation was found homozygously in two infants, it resulted in severe antenatal multisystemic involvement with no detectable CPT II activity in lymphocytes using the forward assay;[10] in contrast, when found heterozygously (S113L and 413delAG-F448L), it led to the muscle form in all patients described (this study,[7]).

Symptoms of muscle form CPT II deficiency usually develop during prolonged exertion when the oxidation of long-chain fatty acids is the main energy source. All patients exhibited normal total CPT activity but abnormal inhibition of CPT II by malonyl-CoA, palmitoyl-CoA, palmitoylcarnitine, Tween 20, and Triton X-100. Malonyl-CoA and palmitoyl-CoA are naturally occurring regulators of this pathway, suggesting that regulation, but not catalytic activity, of the enzyme is impaired.[4]

Structural modeling of a highly conserved region of CPT II predicted a membrane anchor, indicating only a partial insertion of CPT II into the membrane.

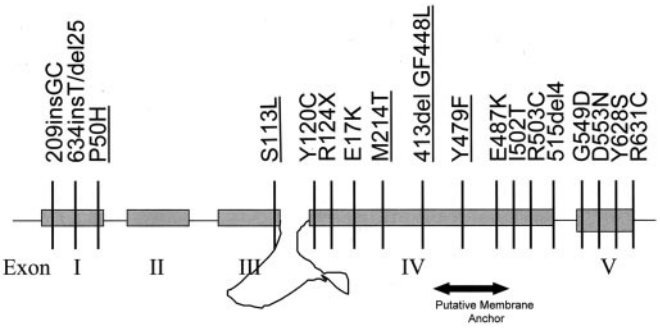

Figure 2. Distribution of all known mutation associated with the muscle form of CPT II deficiency. Mutations present in our patient sample are underlined.

Table *Multiple sequence alignment of highly conserved domains of acyltransferases from different species:Homology plot of the regions containing the newly identified mutations in this study**

Enzyme	Species	F448L	Y479F	M214T
CPT II	Human	DCVQ F QRGGKE	AFLRQ Y GQTVAT	NAYPLD M SQYFRL
CPT II	Rat	DSIQ F QRGGK	AFLRQ Y GQTVAT	NAYPLD M SQYFRL
CAT	Human	ITVMV F HHFGK	AYYRI Y GQACA	K-PLCM M NQYYQ
CHAT	Rat	IVYK F DNYGK	AYYRL Y QRLVPT	GQ-PLC M KQYYRLF
CHAT	Mouse	FIVYK F DNYGK	AYYRL Y QRLVPT	GQ-PLC M KQYYRLF
CHAT	Pig	DFTVYK F DDYGK	AFYRL H GRFVPT	SGQPLC M KQYYGLF
COT	Rat	IAASTV F TSFGK	AYYRL H GRPGCC	NTPLD M NQFRML
CPT I	Rat	LHSFP F DSFGK	IPLC S AQWERL	LAHYKD M GKFCLT

* The amino acids exchanged in our patients are highly conserved, indicating functional importance.

Accession codes: CPT II human = Swiss-Prot P23786; CPT II rat = Swiss-Prot P18886; CAT human = EMBL X78706; COT rat = Swiss-Prott P11466; CHAT = ratSwiss-Prott P32738; CHAT mouse = Swiss-Prott Q03059; CHAT pig = Swiss-Prott P13222; CPT I rat = Swiss-Prott P32198.

CPT = carnitin palmitoyltransferase; CAT = carnitin acetyltransferase; CHAT = cholin acetyltransferase; COT = carnitin octanoyltransferase.

This is consistent with a relatively loose association of the protein with the inner mitochondrial membrane in rats. The observation that the detergents Tween 20 and Triton X-100, which disrupt membranes, lead to reduced CPT activity in patients may be explained by an altered tertiary structure in mutant CPT II when membrane association is lost.

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