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Molecular characterization of inherited carnitine palmitoyltransferase II deficiency

(fatty acid/mutation/hypoketotic hypoglycemia/myopathy/polymerase chain reaction)

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ABSTRACT **Deficiency of carnitine palmitoyltransferase II (CPTase II; palmitoyl-CoA:L-carnitine *O*-palmitoyltransferase, EC 2.3.1.21) is a clinically heterogeneous autosomal recessive disorder of energy metabolism. We studied the molecular basis of CPTase II deficiency in an early-onset patient presenting with hypoketotic hypoglycemia and cardiomyopathy. cDNA and genomic DNA analysis demonstrated that the patient was homozygous for a mutant CPTase II allele (termed *ICV*), which carried three missense mutations: a G-1203 → A transition, predicting a Val-368 → Ile substitution (V368I); a C-1992 → T transition, predicting an Arg-631 → Cys substitution (R631C); and an A-2040 → G transition, predicting a Met-647 → Val substitution (M647V). Genomic DNA analysis of family members showed that the mutations cosegregated with the disease in the family. However, screening of 59 healthy controls demonstrated that both the V368I and M647V mutations are sequence polymorphisms with allele frequencies of 0.5 and 0.25, respectively. By contrast, the R631C substitution was not detected in 22 normal individuals or in 12 of 14 CPTase II-deficient patients with the adult muscular form. Notably, 2 adult CPTase II-deficient patients were heterozygous for the *ICV* allele, thus suggesting compound heterozygosity for this and a different mutant allele. The consequences of the three mutations on enzyme activity were investigated by expressing normal and mutated CPTase II cDNAs in COS cells. The R631C substitution drastically depressed the catalytic activity of CPTase II, thus confirming that this is the crucial mutation. Interestingly, the V368I and M647V substitutions, which did not affect enzyme activity alone, exacerbated the effects of the R631C substitution. Biochemical characterization of mutant CPTase II in patient's cells showed that the mutations are associated with (*i*) severe reduction of V_{max} (≈90%), (*ii*) normal apparent K_m values, and (*iii*) decreased protein stability.**

The carnitine palmitoyltransferase (CPTase; palmitoyl-CoA:L-carnitine *O*-palmitoyltransferase, EC 2.3.1.21) enzyme system, in conjunction with the acyl-CoA synthetase and the carnitine/acylcarnitine translocase, provides the mechanism whereby long-chain fatty acids are transferred from the cytosolic compartment to the mitochondrial matrix to undergo β-oxidation (1, 2). According to the current working model, the CPTase system consists of two mitochondrial membrane-bound enzymes, CPTase I and CPTase II. CPTase I is located on the inner side of the outer mitochondrial membrane, is specifically inhibited by malonyl-CoA, and loses activity upon exposure of mitochondria to strong detergents. By contrast, CPTase II is located on the inner mitochondrial membrane, is insensitive to malonyl-CoA inhibition, and is released in active form upon exposure of mitochondria to a variety of detergents (3, 4). Furthermore, CPTase I exists as tissue-specific isoforms, whereas CPTase II does not (4).

Because of the pivotal role played by CPTase I and CPTase II in the fatty acid oxidation pathway, a genetic defect of one of these enzymes (McKusick nos. 255110 and 255120; see ref. 5) has major clinical consequences. Classically, recessively inherited CPTase II deficiency presents in young adults with recurrent episodes of paroxysmal myoglobinuria triggered by prolonged exercise, cold, or fever (6, 7). Although the enzyme defect is evident in tissues other than skeletal muscle (6, 8), the disease usually presents only with the muscular symptomatology. More recently, though, CPTase II deficiency has been observed also in children and newborns presenting with hypoketotic hypoglycemia, cardiomyopathy, and sudden death (9–12).

These observations indicate that CPTase II deficiency is a complex disorder with phenotypic heterogeneity that may reflect underlying heterogeneity at the molecular level. As a first step to understanding the molecular basis of CPTase deficiency, we have cloned the full-length cDNA of human CPTase II (13, 14). We now report the identification and characterization of the molecular defect in a patient with the early-onset form of CPTase II deficiency presenting with hypoketotic hypoglycemia and cardiomyopathy.

PATIENTS AND METHODS

Patients and Cell Lines. The proband D.M. (cell line F278), age 5 yr, was a member of a family (M.) of southern Italian ancestry. He and his healthy sister, age 8 yr, were the progeny of first-cousin parents. He suffered from recurrent episodes of vomiting, sweating, and lethargy. At age 23 months, he had an acute episode of seizures, coma, and respiratory distress, which led ultimately to severe brain damage. Physical examination revealed moderate hepatomegaly. Two-dimensional echocardiography showed a mild dilated cardiomyopathy with left atrial and ventricular enlargement but no signs of systolic dysfunction. A 17-hr fast revealed mild hypoglycemia without ketone body production. Oxidation of palmitate was decreased (<25% of normal), but oxidation of butyrate and octanoate was normal. Long chain, medium chain, and short chain acyl-CoA dehydrogenases were normal. CPTase I activity (measured by assay A in ref. 7) was normal, whereas CPTase II activity (measured by assay B in ref. 7) was reduced to 16.4%, 8.8%, and 6.6% of normal control in fibroblasts, lymphoblasts, and skeletal muscle, respectively (see Fig. 4*A*). Fibroblast CPTase II activity was also reduced by 40% and 35% in the father and in the mother, respectively.

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Abbreviations: CPTase, carnitine palmitoyltransferase; nt, nucleotide(s).

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Fibroblasts, lymphoblasts, or peripheral blood leukocytes were also collected from 59 unrelated controls and from 14 unrelated CPTase II-deficient patients with the adult muscular form [F20 (7, 8), F382 (7, 15), F63, F85, F123, F191, F437, F484, 91/3314, H131, T3, T7, T10, and T27]. All of the subjects studied were Caucasian, and most were from Italy.

PCR Amplification and Analysis of CPTase II cDNA. Isolation of fibroblast poly(A)$^+$ mRNA and cDNA synthesis were performed with commercial kits (Invitrogen, San Diego) according to the manufacturer's instructions. pdN_6 were used to prime reverse transcription. The entire CPTase II cDNA coding region [nucleotides (nt) 102–2078] plus some untranslated 5′ and 3′ flanking regions (13) was amplified by PCR (16) in five 330- to 750-base-pair (bp) overlapping fragments (Fig. 1). The PCR (94°C for 2 min, 60°C for 2 min, and 72°C for 2 min) was carried out for 35 cycles with the primers (Fig. 1) at a concentration of 0.2 μM.

PCR-amplified cDNA fragments were subcloned into the *Sma* I-digested pGEM-7Zf(+) vector (Promega) and sequenced by the dideoxynucleotide chain-termination method (17). Sequence differences were evaluated by sequence analysis of five to eight independent subclones per PCR fragment and by direct sequencing (18) of PCR products.

PCR Amplification and Analysis of Genomic DNA. Genomic DNA was prepared as described (19). Two sites were amplified, a 117-bp region (nt 1965–2081) encompassing the C-1992 → T and the A-2040 → G mutations using the paired primers G and H (Fig. 1) and a 108-bp region (nt 1122–1229) encompassing the G-1203 → A mutation using the paired primers D1 and F (Fig. 1). The C-1992 → T and the A-2040 → G mutations create a *Bbv* I site (N_8GC<u>T</u>GC) (see Fig. 2*B*) and a *Mae* II site (AC<u>G</u>T), respectively. Because the G-1203 → A transition causes no restriction-site change, an artificial *Bst*XI site (CC<u>A</u>N_6TGG) was introduced into the mutant sequence by substituting A-1211 with C in the antisense primer F [5′-ATCACCCCAAGAGTGCTC<u>C</u>AAGTGGA-3′ (substitution is underlined)]. The PCR products were digested with the appropriate restriction enzyme and then analyzed on a 5% agarose gel (3.75% NuSieve GTG/1.25% SeaKem; FMC). The mutant sequences were identified by the following digestion patterns: *Bbv* I, 104 and 13 bp; *Mae* II, 74 and 43 bp; *Bst*XI, 87 and 21 bp.

Plasmid Construction and Transfection of Mammalian Cells. A full-length normal cDNA clone (pG7C1-WT) containing the entire coding region of human CPTase II was reconstructed by three-part ligation of the 5′ and 3′ *Eco*RI fragments of clone L60 (13) into the *Eco*RI-digested pGEM-7Zf(+) vector. Plasmid pG7C1-WT was used as a parental vector to generate three full-length mutant CPTase II cDNA clones, plasmid pG7C1-ICV [Val-368 → Ile/Arg-631 → Cys/Met-647 → Val (V368I/R631C/M647V) triple mutant], plasmid pG7C1-C (R631C mutant), and plasmid pG7C1-IV (V368I/M647V double mutant). The mutations were cloned into pG7C1-WT using a 532-bp *Sty* I cassette (nt 1184–1715) and a 487-bp *Eag* I/*Hin*dII cassette (nt 1590–2076) derived from subclones of the patient's cDNA. An expression plasmid clone containing the normal CPTase II cDNA (pcDC1-WT) was constructed by subcloning the 2.2-kilobase *Xba* I/Klenow/*Bam*HI fragment derived from pG7C1-WT into the *Hin*dIII/Klenow/*Bam*HI-digested expression vector pcDNA I (Invitrogen) (20). The mutant expression clones pcDC1-ICV, pcDC1-C, and pcDC1-IV were constructed likewise.

Liposome-mediated transfection was performed essentially as described (21). COS-1 cells were transfected in 10-cm Petri dishes with 3 ml of Opti-MEM medium (GIBCO) containing 3 μg of each plasmid DNA and 40 μg of Lipofectin (BRL). To normalize for any variability in transfection efficiency, plasmid pSV-βGal (Promega), an expression vector containing the *Escherichia coli* β-galactosidase cDNA, or plasmid pODb, an expression vector containing the human ornithine transcarbamoylase cDNA [gift of A. Horwich, Yale University (22)], was cotransfected as an internal standard. Mock transfection was carried out with control plasmid alone (pSV-βGal or pODb).

Enzyme Assays and Immunoprecipitation. Cell extracts were assayed for CPTase II enzyme activity as described (forward assay A or backward assay B in ref. 7). The levels of exogenous CPTase II activity—i.e., residual activity following subtraction of endogenous activity—in COS-1 cells transfected with various mutant CPTase II cDNAs or the normal CPTase II cDNA were normalized for β-galactosidase activity. β-Galactosidase activity was determined as described (23). Protein was measured by a dye-binding method (kit from Bio-Rad).

Anti-CPTase II antibodies were raised against a chemically synthesized 44-residue-long peptide (residues 567–610). Glutaraldehyde coupling of the peptide to keyhole limpet hemocyanin (Calbiochem) and immunization protocols were as described (24).

Fibroblasts and COS-1 cells were pulse-labeled with L-[^{35}S]methionine as described (25). COS-1 cells were harvested and mitochondria were prepared as described (26). Isolated mitochondria were further fractionated into membranes and matrix preparations essentially as described (4). Cell extracts and submitochondrial fractions were solubilized and immunoprecipitated (25) with anti-CPTase II antiserum. The resulting supernatant was immunoprecipitated with anti-ornithine transcarbamoylase antiserum [gift of F. Kalousek, Yale University (22)] or anti-medium chain acyl-CoA dehydrogenase antiserum (27).

RESULTS AND DISCUSSION

Sequence Analysis of Mutant CPTase II cDNA. The F278 CPTase II sequence differed from the published reference CPTase II sequence (13, 14) by 3 nt substitutions, a G → A transition at nt 1203, a C → T transition at nt 1992, and an A → G transition at nt 2040. These differences were detected in all the clones examined. Two substitutions, G-1203 → A and A-2040 → G, would result in conservative amino acid changes—namely V368I and M647V, respectively. Direct sequenc-

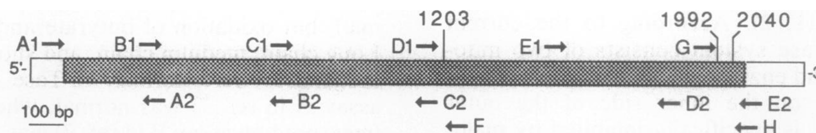

FIG. 1. CPTase II cDNA with the location of the primers used for PCR amplification. Stippled box, coding region. Positions of the G-1203 → A, the C-1992 → T, and the A-2040 → G transitions are shown. Oligonucleotide primers were based on the published CPTase II cDNA sequence (13, 14). Reverse-transcribed cDNA was amplified with five sets of primers (A1 and A2, B1 and B2, C1 and C2, D1 and D2, E1 and E2). Primers A1, B1, C1, D1, and E1 corresponded to nt 39–63, nt 320–343, nt 701–724, nt 1122–1144, and nt 1497–1519, respectively. Primers A2, B2, C2, D2, and E2 were complementary to nt 351–376, nt 729–752, nt 1152–1178, nt 1857–1879, and nt 2091–2115, respectively. Genomic PCRs were carried out with two sets of primers (D1 and F, G and H). Primer G corresponded to nt 1965–1991 and primer H was complementary to nt 2055–2081 of the cDNA sequence. In the antisense primer F encompassing positions 1204–1229 in the cDNA, A-1211 was artificially substituted with C (see *Patients and Methods*).

ing of the amplification fragments confirmed both mutations and suggested that they were homozygous (results not shown). The potentially harmful point mutation was the C → T transition at position 1992 (R631C), which changes the CGC codon 631 for arginine—a bulky cationic residue—to a TGC codon for cysteine—a more compact neutral amino acid (Fig. 2A). To confirm that the substitution was not due to PCR or cloning artifacts, direct sequencing of the amplification fragment E was performed. Sequence analysis demonstrated a single species of abnormal CPTase II cDNA, thus suggesting that the mutation was homozygous (Fig. 2B).

Analysis of Genomic DNAs of the Patient and Family Members. To confirm that the G-1203 → A, C-1992 → T, and A-2040 → G transitions were present in both alleles of patient F278 and to determine whether the mutations cosegregated with CPTase II deficiency, genomic DNAs isolated from the patient and available family members were analyzed by PCR and restriction enzyme digestion.

Bbv I digestion of the PCR fragments was used to detect the C-1992 → T transition (Fig. 3). The PCR products from the control (lane N) and the patient's non-affected sibling (lane 4) were totally resistant to the enzyme, suggesting homozygosity for the normal allele. In contrast, the PCR product from the patient (lane 3) was completely digested, thus indicating homozygosity for the mutant allele. Furthermore, digestion of the amplified DNA from the clinically unaffected parents (lanes 1 and 2) gave both the 117-bp fragment and the smaller fragment of 104 bp, consistent with the presence of both the mutated and the normal allele.

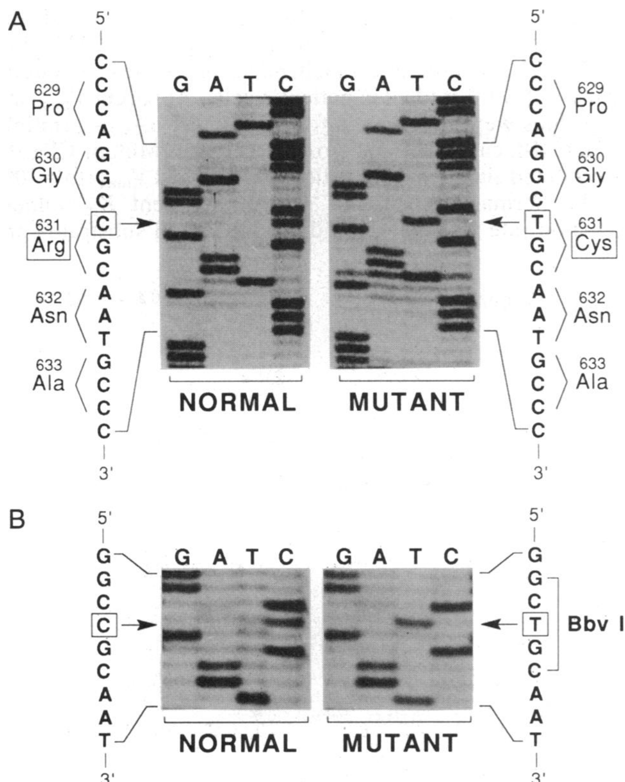

FIG. 2. Partial nucleotide sequences of the CPTase II cDNAs from a normal individual and from patient F278 showing the R631C mutation. (*A*) Amplified fragment E (see text) was subcloned, and five clones were sequenced. The sequence from positions 1983–1999 is shown. (*B*) Direct sequencing of amplified fragment E. The sequence from positions 1989–1997 is shown. C-1992 in the normal sequence is substituted with T in the mutant (arrows). Substituted nucleotide and its normal counterpart are boxed. The sequence is labeled 5′ and 3′ in reference to the orientation of the CPTase II cDNA (13).

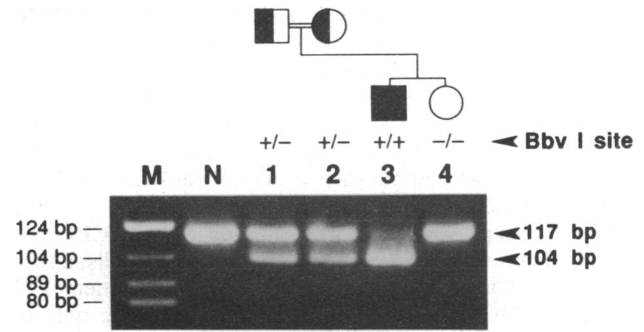

FIG. 3. Detection of the R631C mutation by *Bbv* I cleavage of PCR-amplified genomic DNAs of patient F278 and family members. Partial pedigree of the patient's family and *Bbv* I digestion of PCR products are shown. The 117-bp fragments were digested with *Bbv* I and analyzed on a 5% agarose gel. In the presence of the C-1992 → T transition, *Bbv* I digestion generates a smaller fragment of 104 bp. Lanes: M, molecular size markers; N, normal control; 1, father; 2, mother; 3, patient; 4, nonaffected sibling. +, Positive for *Bbv* I site; −, negative for *Bbv* I site.

The G-1203 → A and the A-2040 → G transitions were analyzed by PCR/*Bst*XI and PCR/*Mae* II digestion, respectively (see *Patients and Methods*). The digestion patterns (not shown) confirmed that the patient was homozygous for both mutations, that the parents were heterozygous, and that the nonaffected sibling was homozygous for both normal alleles.

The results obtained thus far established the existence of a mutant CPTase II allele—designated allele *ICV*—which contained the V368I, R631C, and M647V substitutions and cosegregated with the disease in this family.

Frequency of the R631C Mutation Among Normal Individuals and CPTase II-Deficient Patients. *Bbv* I digestion was performed with amplified genomic DNA from 22 unrelated controls and 14 unrelated CPTase II-deficient patients with the adult muscular form (results not shown). The *Bbv* I site created by the R631C mutation was not detected in any of the controls. Furthermore, it was not observed in 12 of 14 CPTase II-deficient patients. Notably, two patients (F20 and 91/3314) were heterozygous for the R631C mutation. In both cases, *Bbv* I, *Bst*XI, and *Mae* II analysis of PCR-amplified DNA from the patients and the asymptomatic parents indicated that the patients, homozygous for both the V368I and M647V mutations, had inherited the *ICV* allele from the mother and, presumably, an as yet unidentified mutant allele from the father—i.e., they might be tentatively identified as compound heterozygotes for two different mutations. These results (*i*) clearly demonstrate that the R631C mutation is not a common substitution at the CPTase II locus, (*ii*) suggest that this mutation is the disease-causing mutation in patient F278, and (*iii*) lend support to the hypothesis that there is a molecular basis for the phenotypic heterogeneity in the disease.

V368I and M647V Mutations Represent Sequence Polymorphisms. Because the point mutations at positions 1203 (V368I) and 2040 (M647V) cosegregated with the disease in the original patient's family, we screened 59 clinically asymptomatic unrelated Caucasians—22 of whom had also been screened for the R631C mutation—for both mutations by PCR/*Bst*XI and PCR/*Mae* II digestion as described above. Six genotypes were identified: $V368I^{-/-}M647V^{-/-}$ (12/59), $V368I^{-/+}M647V^{-/-}$ (18/59), $V368I^{-/+}M647V^{-/+}$ (17/59), $V368I^{+/+}M647V^{-/-}$ (4/59), $V368I^{+/+}M647V^{-/+}$ (4/59), and $V368I^{+/+}M647V^{+/+}$ (4/59). The observed allele frequencies for the V368I and the M647V mutations were 0.5 and 0.25, respectively, and both variants exhibited Hardy–Weinberg equilibrium. These results clearly establish that both V368I

and M647V substitutions are polymorphisms. The possibility exists, however, that the presence of the V368I and/or M647V mutations exacerbates the effects of the R631C mutation or, conversely, that the two polymorphisms are in some way helpful, mitigating the effects of a deleterious mutation. We investigated these issues by expressing the wild-type and mutant CPTase II cDNA constructs in transfected COS cells.

***In Vitro* Expression of Wild-Type and Mutant CPTase II cDNAs.** Three cDNAs were expressed in COS-1 cells: a triple mutant containing the mutations identified in patient F278 (V368I, R631C, and M647V), a mutant containing only the putative disease-causing mutation R631C, and a double mutant containing the two polymorphisms V368I and M647V. Although endogenous CPTase II activity was present in mock-transfected COS-1 cells (mean ± SD of 10 dishes, 29.0 ± 4.4 nmol of [*methyl*-^{3}H]carnitine released per min per mg), expression of wild-type cDNA (pcDC1-WT) resulted in a 3.7 ± 1.3-fold induction of CPTase II activity (105.8 ± 39.6 nmol per min per mg; $n = 10$), which is similar to that reported for the rat enzyme (26). The results of the transfection experiments are shown in Fig. 4A (histogram 2). The activities in cells transfected with pcDC1-ICV, pcDC1-C, and pcDC1-IV were 14.7% ± 4.0% (mean ± SD; $n = 5$), 31.7% ± 6.4% ($n = 6$), and 86.4% ± 9.0% ($n = 4$) of the activity in pcDC1-WT-transfected cells, respectively. Double mutant-transfected cells exhibited slightly reduced relative activity. By contrast, both the triple mutant and the single mutant exhibited a significant reduction of enzyme activity ($P \leq 10^{-4}$). Although the relative activities were very similar between triple mutant-transfected cells and the patient's tissues (Fig. 4A, histogram 1), the relative activities in pcDC1-ICV- and pcDC1-C-transfected cells were significantly different ($P = 10^{-3}$).

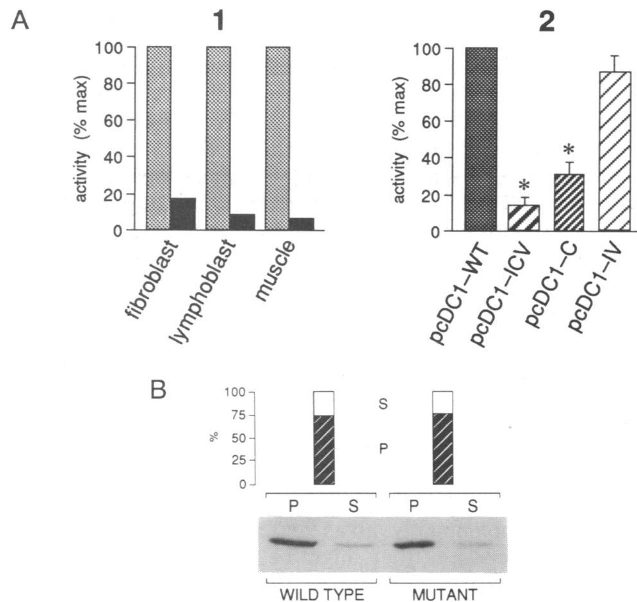

FIG. 4. CPTase II expression in patient's fibroblasts, lymphoblasts, and muscle, and in transfected COS-1 cells. CPTase II activity was measured in the backward direction (assay B in ref. 7). (*A*) Histogram 1 shows relative CPTase II activities expressed in tissues from a normal control (stippled bars) and from patient F278 (solid bars). Histogram 2 shows relative exogenous CPTase II activities in COS-1 cells transfected with wild-type and mutant expression vectors. Level of exogenous CPTase II activity in cells transfected with mutant CPTase II cDNAs is expressed as a percentage of the level in cells transfected with normal CPTase II cDNA. Bars and vertical lines indicate means ± SD of four (pcDC1-IV), five (pcDC1-ICV), or six (pcDC1-C) experiments. *, Significant difference ($P \leq 10^{-4}$) between mutant and wild-type means. (*B*) SDS/PAGE analysis of CPTase II immunoprecipitated from submitochondrial fractions from pulse-labeled cDNA-transfected COS-1 cells. COS-1 cells were transfected with pcDC1-WT, indicated as wild type, or pcDC1-ICV, indicated as mutant, as described above except that pODb was substituted for pSV-βGal. Histogram above the gel shows relative amounts of radiolabeled CPTase II in the membrane-containing pellet fraction (P) and soluble matrix-containing fraction (S), as estimated by densitometric analysis of fluorograms.

Expression of normal and mutant cDNAs in transfected COS-1 cells was further investigated by immunoprecipitation studies. Mitochondria were isolated from radiolabeled transfected cells, fractionated into a membrane-containing fraction and a soluble matrix-containing fraction, and then immunoprecipitated (Fig. 4B). First, the reduction of CPTase II activity in the mutant-transfected cells was not caused by a failure of production of the enzyme. Second, most of both normal and mutant CPTase II was detected in the mitochondrial membrane-containing fraction (4), thus suggesting normal topography of the mutant protein.

These results clearly demonstrate that (*i*) the R631C substitution is the crucial disease-causing mutation in this patient and that (*ii*) the V368I and M647V substitutions represent sequence polymorphisms that alone do not substantially affect CPTase II but that acquire functional significance in the presence of the R631C substitution. It remains to be established whether this complex mutant represents a prototype for a larger group of CPTase II mutants in which the V368I and M647V substitutions might offer an unfortunate structural background, exacerbating the effects of an additional mutation.

Biochemical Characterization of Patient F278 Homozygous for Allele *ICV*. To elucidate the mechanism by which the amino acid substitutions identified in patient F278 alter CPTase II function, the biochemical properties of mutant CPTase II were studied in patient's cells. Apparent V_{max} and K_m values were determined for palmitoyl-CoA, L-carnitine, palmitoyl-L-carnitine, and CoASH (Fig. 5). Mutant CPTase II exhibited significant reduction of estimated V_{max} (to ≈10% of the normal control) but normal apparent K_m values. Reaction rate was markedly depressed at all substrate con-

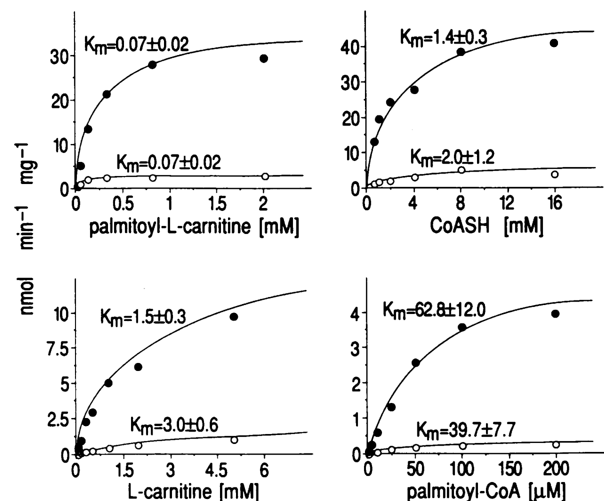

FIG. 5. Substrate-dependent kinetics of CPTase II in lymphoblast homogenates of patient F278 (○) and a normal control (●). Lymphoblasts were cultured in α-minimal medium and supplemented with 15% (vol/vol) fetal calf serum without antibiotics. Assays were carried out as described (forward assay A or backward assay B in ref. 7). Apparent K_m was obtained by changing one of the substrates. Concentrations of the other substrates were fixed at the levels of the standard assay conditions (7). Apparent Michaelis constants were determined by using a computer program based on nonlinear regression analysis (28). Values represent means ± SE of three determinations.

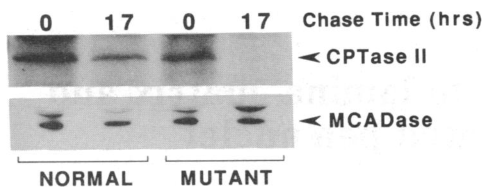

FIG. 6. Stability of CPTase II in cultured fibroblasts from patient F278 and a normal control. Cells were pulse-labeled with L-[^{35}S]methionine for 2 hr and then chased for 17 hr as described (25). Solubilized cells were immunoprecipitated (25) with anti-CPTase II antiserum and the resulting supernatant was immunoprecipitated with anti-MCADase (medium chain acyl-CoA dehydrogenase) antiserum (27). Immunoprecipitates were analyzed by SDS/PAGE. Fluorograms of gels are shown. Chase time is indicated above the gels in hours.

centrations. Thus, the kinetic properties of this mutant CPTase II are different from those described in a previous study (29), which provided evidence that in some patients with muscular CPTase II deficiency the mutant enzyme exhibited normal activity under optimal assay conditions but was abnormally inhibited by increasing concentrations of either palmitoyl-CoA or palmitoyl-L-carnitine.

Metabolic labeling of patient's fibroblasts in a pulse-chase protocol demonstrated normal biosynthesis of a normal-sized CPTase II monomer that appeared less stable than the normal counterpart (Fig. 6). A time course experiment (data not shown) demonstrated that the half-life of the mutant enzyme was considerably shorter ($t_{1/2} \leq 5$ hr) than that of the normal protein ($t_{1/2} \approx 14$ hr).

In the absence of structural data, it is impossible to predict the effects of amino acid changes on structure and function of CPTase II. Kinetic analysis indicates that the mutations in allele *ICV* do not affect substrate binding sites. This perhaps suggests more complex structural alterations. Like most mitochondrial proteins, CPTase II is synthesized in the cytosol as a larger precursor, translocated into mitochondria, proteolytically processed to the mature subunit, and assembled into the tetrameric form. It has been demonstrated that, in mitochondria, proteins with abnormal conformations are degraded more rapidly than normally folded counterparts (30). Improper folding or assembly of multimeric proteins also results in accelerated degradation of the free subunits (22, 30). Thus, the observed increased susceptibility of this mutant CPTase II to proteolysis may suggest incomplete folding and/or assembly of the mutant enzyme in this patient.

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