



The mitochondrial carnitine/acylcarnitine carrier: Function, structure and physiopathology

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ABSTRACT

The carnitine/acylcarnitine carrier (CAC) is a transport protein of the inner mitochondrial membrane that belongs to the mitochondrial carrier protein family. In its cytosolic conformation the carrier consists of a bundle of six transmembrane α -helices, which delimit a water filled cavity opened towards the cytosol and closed towards the matrix by a network of interacting charged residues. Most of the functional data on this transporter come from studies performed with the protein purified from rat liver mitochondria or recombinant proteins from different sources incorporated into phospholipid vesicles (liposomes). The carnitine/acylcarnitine carrier transports carnitine and acylcarnitines with acyl chains of various lengths from 2 to 18 carbon atoms. The mammalian transporter exhibits higher affinity for acylcarnitines with longer carbon chains. The functional data indicate that CAC plays the important function of catalyzing transport of acylcarnitines into the mitochondria in exchange for intramitochondrial free carnitine. This results in net transport of fatty acyl units into the mitochondrial matrix where they are oxidized by the β -oxidation enzymes. The essential role of the transporter in cell metabolism is demonstrated by the fact that alterations of the human gene SLC25A20 coding for CAC are associated with a severe disease known as carnitine carrier deficiency. This autosomal recessive disorder is characterized by life-threatening episodes of coma induced by fasting, cardiomyopathy, liver dysfunction, muscle weakness, respiratory distress and seizures. Until now 35 different mutations of CAC gene have been identified in carnitine carrier deficient patients. Some missense mutations concern residues of the signature motif present in all mitochondrial carriers. Diagnosis of carnitine carrier deficiency requires biochemical and genetic tests; treatment is essentially limited to important dietetic measures. Recently, a pharmacological approach based on the use of statins and/or fibrates for the treatment of CAC-deficient patients with mild phenotype has been proposed.

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1. Introduction

Oxidation of fatty acids in mitochondria coupled to oxidative phosphorylation is the most important pathway for the production of metabolic energy during fasting. This process occurs in the mitochondrial matrix where the enzymes of fatty acid β -oxidation are located. Fatty acyl groups are transported from the cytosol into the mitochondrial matrix by means of the carnitine shuttle system (Fig. 1). In the cytosol, fatty acid units are transferred from acyl-CoAs to carnitine by the action of the carnitine-palmitoyl-transferase 1 (CPT1) which is located on the external surface of the outer mitochondrial membrane (Lee et al., 2011; Rufer et al., 2009); the formed acylcarnitines cross the outer membrane, which is permeable to small molecules (Zeth and Thein, 2010) and are translocated through the inner mitochondrial membrane by the carnitine/acylcarnitine carrier (CAC) in exchange for intramitochondrial free carnitine; in the mitochondrial matrix, fatty acyl units are transferred from carnitine to matrix CoA by carnitine-palmitoyl-transferase 2 (CPT2) and the formed mitochondrial acyl-CoAs are oxidized by the β -oxidation enzymes. Therefore, the molecular components of the carnitine shuttle system, carnitine acyl transferases and CAC, are essential for the mitochondrial oxidation of fatty acids and, hence, for life (Ramsay et al., 2001). Most of the functional and structural properties of the mitochondrial CAC transporter have been elucidated after the characterization of the carnitine acyl transferases, as usually occurs for membrane proteins with respect to soluble enzymes. This review focuses on the molecular aspects of CAC which are connected with human pathology.

2. Function and structure of CAC

2.1. Functional properties of CAC and regulation of its transport activity

Mitochondrial carnitine transport has been preliminarily studied using isolated intact mitochondria (Murthy and Pande, 1984 and references therein). However, most of the available knowledge on the function of the mitochondrial CAC derives from studies performed in liposomes reconstituted with CAC purified from rat liver mitochondria (Indiveri and Palmieri, 1989; Indiveri et al., 1990) or recombinant CAC proteins obtained by gene expression in *Escherichia coli* or *Saccharomyces cerevisiae* (Indiveri et al., 1998; Palmieri et al., 1999; De Lucas et al., 2008). In these studies the purified CAC was inserted into the lipid bilayer of liposomes (Palmieri et al., 1995). In particular, the procedure of cyclic detergent removal adopted for the reconstitution of CAC allowed the insertion of the purified protein into the artificial membrane with the same orientation displayed by CAC in the native membrane. This procedure is based on a slow removal of the detergent from micelles constituted by purified CAC, detergent and phospholipids. The resulting proteoliposomes are used to study the CAC-mediated transport of carnitine and its acyl derivatives following the flux of [^3H]carnitine from the outside to the inside of the vesicles or vice versa (Fig. 2). Besides carnitine, the transporter accepts acylcarnitines with carbon chain length from 2 to 18 carbon atoms (Indiveri et al., 1990). The mammalian CAC has a higher affinity for long chain acylcarnitines, whereas the fungal transporter for short chain acylcarnitines (De Lucas et al., 2008). CACs from different sources catalyze both an exchange (antiport) and an uniport (i.e. unidirectional transport) of substrates, which are driven by the concentration gradient of the substrates across the membrane and not by the membrane potential or the pH gradient, as in the case of other mitochondrial carriers (Palmieri and Pierri, 2010a). Furthermore, detailed kinetic studies have shown that CAC follows a ping-pong mechanism, which implies that binary carrier-substrate complexes are formed before the transport reaction occurs (Indiveri et al., 1994), in contrast to a sequential mechanism which would involve a ternary complex of two substrates with the carrier protein (Palmieri et al., 1993).

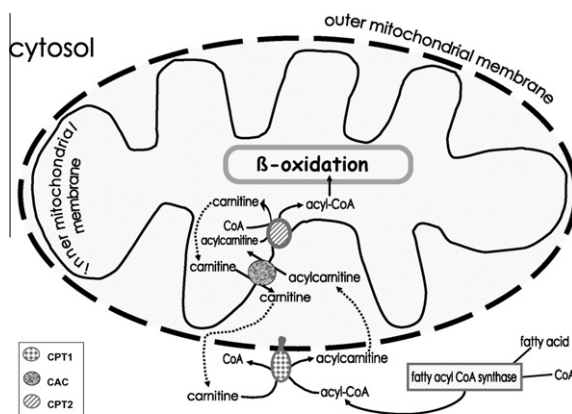


Figure 1

Fig. 1. The mitochondrial carnitine shuttle. Abbreviations: CPT1, carnitine-palmitoyl-transferase 1; CPT2, carnitine-palmitoyl-transferase 2; CAC, carnitine/acylcarnitine carrier.

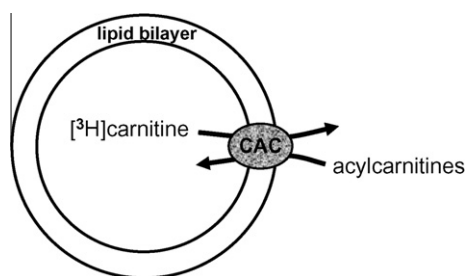


Fig. 2. Schematic representation of the [^3H]carnitine/acylcarnitine exchange (antiport) in proteoliposomes.

In agreement with the ping-pong reaction mechanism of CAC, the activity of the uniport mode of transport has been found to be much lower than that of the exchange reaction (Indiveri et al., 1991a). However, also the uniport transport of carnitine is physiologically important because it is responsible for the net flux of carnitine into mitochondria to equilibrate the matrix level of carnitine with that present in the cytosol. It is worth mentioning that uniport-mediated efflux of carnitine from mitochondria is unlikely to occur during β -oxidation, when the concentration of acylcarnitines is high in the extramitochondrial compartment. The preferred antiport reaction is the entry of acylcarnitines into mitochondria in exchange for carnitine. In fact the activity of the transporter is regulated, among other factors, by the carrier affinities for carnitine and acylcarnitines on the external (cytosolic) and the internal (matrix) side of the mitochondrial membrane. On the external side the K_m for acylcarnitines (in the micromolar range) is much lower than that of carnitine (0.5–1.0 mM) (Indiveri et al., 1991b, 1994). This implies that acylcarnitines are preferentially transported from cytosol into the matrix. In the matrix, acylcarnitines are transformed in acyl-CoA and the exported substrate, under physiological conditions, is carnitine. It should be noted that the average carnitine concentration inside the organelle ranges from 2 to 5 mM (Foster, 2004; Idell-Wenger et al., 1978) and the affinity of carnitine for CAC on the matrix side is about 10 mM (Indiveri et al., 1994). Thus, intramitochondrial carnitine concentration limits the overall rate of the acylcarnitine/carnitine exchange and, hence, represents a regulatory step of fatty acid β -oxidation (Indiveri et al., 1994; Zeth and Thein, 2010; Calvani et al., 2000; Reda et al., 2003). Besides the kinetic aspects of the transport process there are some other important factors that regulate the carrier activity. An example is the absolute requirement of CAC activity for cardiolipin. This was demonstrated by experiments performed with the recombinant rat CAC (Indiveri et al., 1998) produced in *E. coli* which does not synthesize cardiolipin. CAC activation by cardiolipin is a dose–response effect (Giangregorio et al., 2007). Cardiolipin is a specific phospholipid of the mitochondrial inner membrane where its concentration is regulated by its synthesis. Therefore, any modulator of cardiolipin concentration in the mitochondria may indirectly act on the activity of CAC (Paradies et al., 1997). CAC is also strongly regulated by the redox state of its cysteines. Rat and human CAC contains six Cys. It has been found that reagents which bind reversibly (mercurials and methanethiosulfonates) or irreversibly (N-ethylmaleimide) SH groups as well as reagents which induce the formation of disulfides (Cu^{2+} -phenanthroline and diamide) inactivate this transporter (Indiveri et al., 1990, 1992, 1995; Tonazzi et al., 2005; Giangregorio et al., 2007). Thus CAC needs free Cys SH groups in reduced state to be functionally active. Oxidation of these groups may be influenced by the oxidative state of the cell. It is known that Cys residues exposed on the surface of the proteins may serve as redox biosensors of the cells (Dalle-Donne et al., 2007). It is likely that oxidative stress or other factors which generate intracellular reactive oxygen species (ROS) that bind SH groups (Finkel and Holbrook, 2000; Jones, 2006) may affect CAC activity with consequences on energy metabolism. Physiological compounds which regulate the oxidation state of cell thiols, such as glutathione or thioredoxin (Dalle-Donne et al., 2007; Spadaro et al., 2010), may also play an important role.

2.2. Structural properties of CAC

CAC was cloned by PCR using degenerate primers and probes based on partial amino acid sequence information, obtained from direct sequencing of internal peptides of the purified CAC protein from rat (Indiveri et al., 1997). Immediately afterwards, the human CAC cDNA was obtained by PCR using primers based on the rat CAC cDNA (Huizing et al., 1997). Both cDNAs contain an open reading frame of 903 bp coding for a protein of 301 amino acids. Based on this information, CAC orthologs from different species have been predicted and in some cases validated (Fig. 3). CAC belongs to the mitochondrial carrier protein family (Palmieri, 2004; Picault et al., 2004; Palmieri et al., 2006, 2011) which comprises about 50 members in *H. sapiens*. The primary structure of CAC, like that of all the other family members, is made up of three tandemly repeated domains of about 100 amino acids in length; and each domain contains a characteristic signature motif PX[DE]XX[RK]X[RK] and consists of two hydrophobic stretches (spanning the membrane as α -helices) linked by a hydrophobic loop located on the matrix side (Indiveri et al., 1997). The tertiary structure of CAC was derived by homology modeling using the crystallographic structure of the carboxyatractyloside-inhibited, ADP/ATP carrier as template (Pebay-Peyroula et al., 2003). The protein consists of a bundle of six transmembrane α -helices (H1–H6), which line a water-accessible funnel-shaped cavity opened towards the cytosol and closed on the matrix side by a salt-bridge network formed by the charged residues of

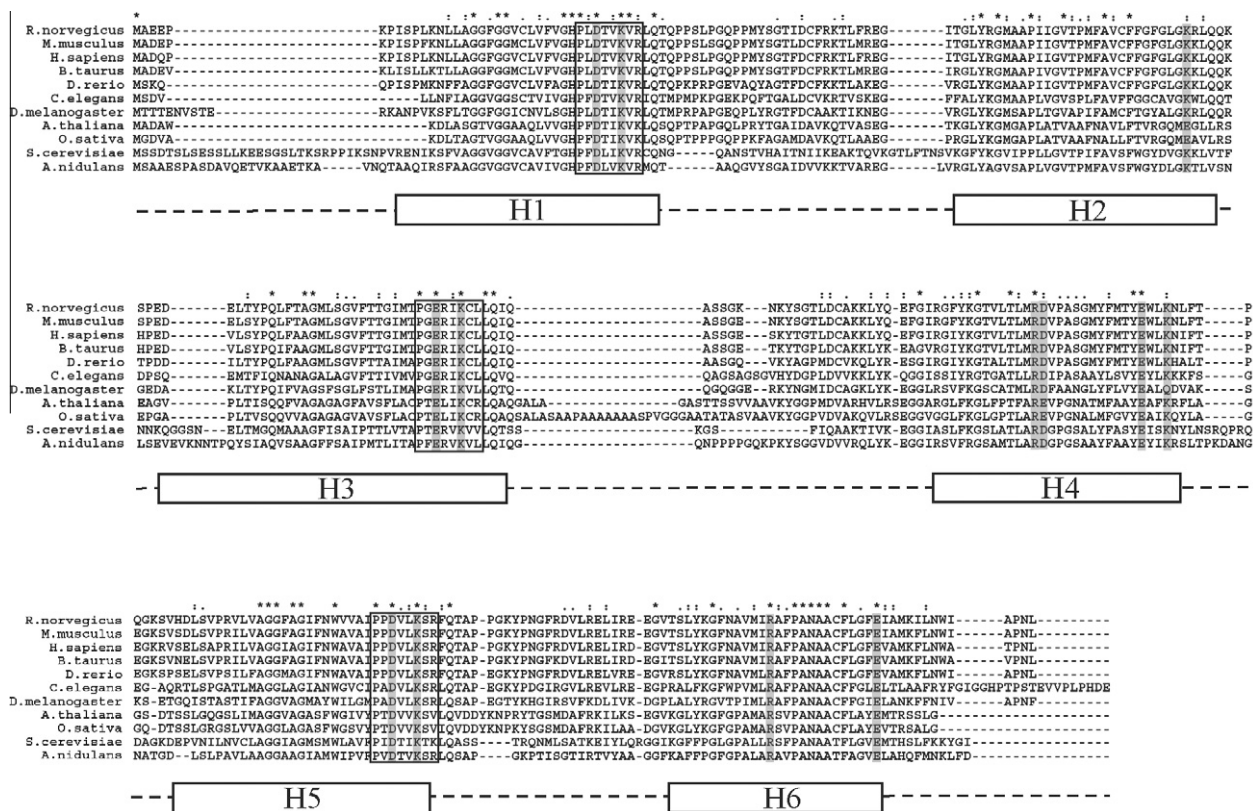


Fig. 3. Alignment of CAC proteins from different sources. CAC proteins (NP446417 from *R. norvegicus*, NP065266 from *M. musculus*, NP000378 from *H. sapiens*, NP001071404 from *B. taurus*, NP957153 from *D. rerio*, NP501223 from *C. elegans*, NP477221 from *D. melanogaster*, NP568670 from *A. thaliana*, NP001065471 from *O. sativa*, NP014743 from *S. cerevisiae* and AJ011563 from *A. nidulans*) were aligned using the Clustal W software. The lower boxes indicate the hydrophobic transmembrane segments H1–H6. Identical amino acids are indicated by asterisks; conservative and highly conservative substitutions are indicated by dots and colons, respectively. The black rectangles highlight the three signature motifs PX[DE]XX[RK]X[RK]. Conserved Lys, Arg, Glu and Asp are highlighted in gray.

the signature motif (Fig. 4). This structure which corresponds to the “c” (cytosolic)-state of the mitochondrial carriers has greatly helped the interpretation of experimental results obtained mainly by site-directed mutagenesis. In these studies one or more amino acids of CAC were mutated and the mutant CAC proteins were tested for activity after their incorporation into liposomes. The first amino acids of CAC which were subjected to site-directed mutagenesis are cysteines, because these residues can be labeled with specific chemical reagents. It was found that each single Cys mutant was substantially active, demonstrating that none of the CAC Cys residues is directly involved in the transport mechanism (Indiveri et al., 2002). Interestingly, SH-blocking reagents failed to inactivate the mutants lacking Cys-136 and Cys-155, in contrast to what observed with the wild-type CAC and the CAC mutants containing Cys-136 or Cys-155 (Indiveri et al., 2002). Thus, Cys-136 and Cys-155 are the target of the SH reagents and in some states of the transport cycle protrude into the translocation path where, when bound by SH-blocking reagents, cause a steric hindrance to the passage of the substrate. Furthermore, CAC mutants lacking Cys-136 or Cys-155 (or both) failed to be inactivated by the disulfide forming reagents diamide or Cu²⁺-phenanthroline (Tonazzi et al., 2005). This means that Cys-136 and Cys-155, which are at a distance of about 10 Å in the c-conformation (Fig. 4), have to become very close (about 2 Å) to form a disulfide. Therefore, the inactivation caused by disulfide formation (cross-linking) between Cys-136 and Cys-155, which only occurs when both these Cys residues are present in the protein, is due to impairment of the carrier conformational changes that are required for substrate translocation to be accomplished. The occurrence of conformational changes during the transport cycle was confirmed by the observation that the accessibilities of Cys-136 and Cys-155 to SH reagents markedly change by increasing the concentration of carnitine inside or outside the proteoliposomes, which, according to the ping-pong mechanism of CAC, favors the m(matrix)-state or the c-state of the carrier, respectively (Giangregorio et al., 2007).

The role of the charged residues of CAC which are highly conserved in the CAC mitochondrial carrier subfamily (Fig. 3) has also been investigated by site-directed mutagenesis. In fact, acid and basic residues should be involved in the recognition/binding of the substrate, which is a zwitterion, and in the transition of the CAC protein between its c- and m-conformations (Fig. 4). These studies have been performed on rat (Tonazzi et al., 2009) as well as on human (De Lucas et al., 2008; Giangregorio et al., 2010) recombinant CAC. The substitution of Lys-35, Glu-132, Arg-178, Asp-179, Asp-231, Lys-234 and

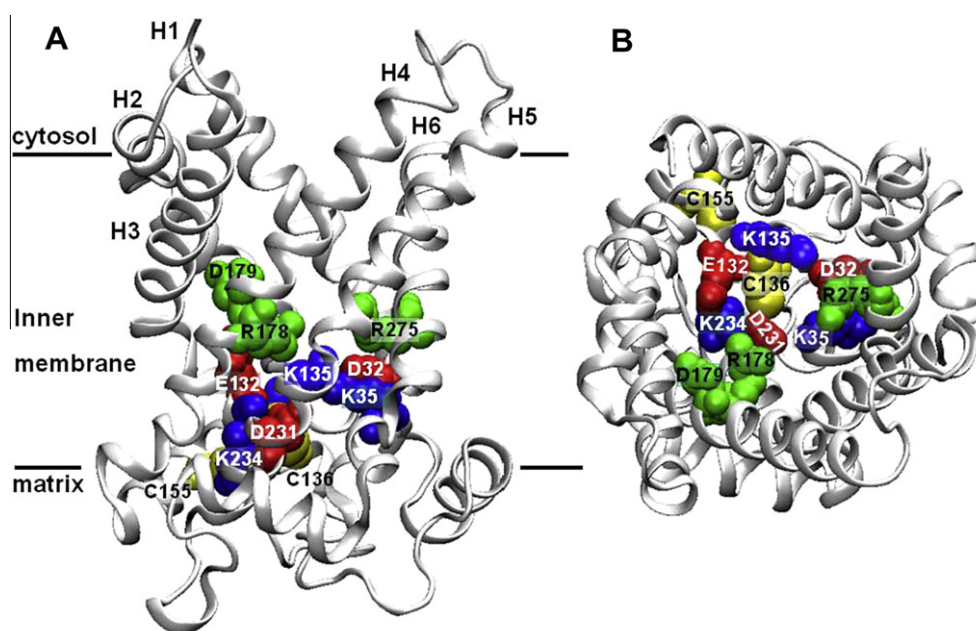


Fig. 4. Critical residues mapped on the structural model of human CAC. The structural model is shown in cartoon with a view from the lateral side (A) and in the cavity from the cytosolic side (B). Transmembrane segments are indicated as H1–H6. R178, D179 and R275 (in green) are residues proposed to be in the substrate binding site; D32, E132 and D231 (in red) and K35, K135 and K234 (in blue) are the residues forming the salt-bridge network on the matrix side; and C136 and C155 (in yellow) are the cysteines which can be labeled by SH-blocking reagents or crossed linked by disulfide-forming reagents during the catalytic transport cycle. The homology structural model has been represented using the molecular visualization program VMD. The inner mitochondrial membrane is represented by lines.

Arg-275 with alanine caused almost complete loss of transport function, whereas conservative replacements lead to a partial (K35R, R178K, D179E, and R275K) or a complete (E132D) recovery of function (Fig. 5). These results show that residues K35, E132, R178, D179, D231, K234 and R275 are essential for CAC transport activity and their electrical charge plays an important role in CAC-mediated transport. In agreement with recent literature concerning mitochondrial carriers (Palmieri and Pierri, 2010b; Kunji and Robinson, 2010; Palmieri et al., 2011), it is likely that the substrate, entered into the carrier cavity from the cytosol, binds at the level of R178, D179 and R275 and this binding triggers a conformational change leading to a temporary opening of the salt-bridge network on the matrix side (matrix gate) allowing the passage of the substrate through the protein into the mitochondrial matrix.

3. CAC gene (SLC25A20)

3.1. Gene structure

Based on the human cDNA sequence the corresponding human CAC gene structure was determined (Iacobazzi et al., 1998). The gene, now named SLC25A20, spans over 42 kb and is split into nine exons. All exon–intron boundaries comply with the GT–AG rule and show a tendency to interrupt the sequence coding for the extramembranous loops rather than the transmembrane α -helices. Using FISH (fluorescence in situ hybridization) the CAC gene was mapped on chromosome 3,p21.31 (Viggiano et al., 1997). Northern blot analysis revealed different levels of expression in human tissues. High levels of CAC transcripts were found in liver, heart and skeletal muscle; much lower levels of CAC transcripts were observed in brain, placenta, kidney, pancreas and lung (Huizing et al., 1998a).

3.2. Functional characterization of the CAC gene promoter

In silico analysis of the SLC25A20 promoter revealed the absence of the TATA box element and the presence of the following elements: the TFB II recognition element (BRE) at –81/–75 bp, the downstream promoter element (DPE) at –18/–13 bp, the peroxisome proliferator-activated receptor element (PPRE) at –99/–80 bp, the forkhead box A (FOXA) at –300/–290 bp and the stimulating protein 1 (Sp1) at –237/–227 bp and –115/–105 bp (Fig. 6) (Iacobazzi et al., 2009; Convertini et al., 2011). DPE and BRE allow binding of the general transcription factors (Convertini et al., 2011). PPRE is bound by PPAR α , a transcription factor which is involved in the regulation of many genes of fatty acid metabolism (Schoonjans et al., 1996) and is the molecular target for several drugs including fibrates and statins, which are widely used in the treatment of

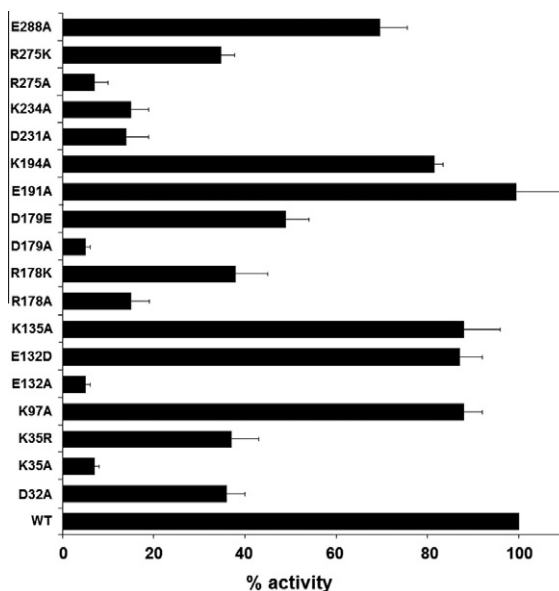


Fig. 5. Transport of carnitine in the proteoliposomes by single-residue replacement CAC mutants. Proteoliposomes were preloaded internally with 15 mM carnitine. Transport was started by the addition of 0.1 mM [^3H]carnitine and terminated after 30 min.

hypercholesterolemias and hyperlipidemias (Huijgen et al., 2008; Wierzbicki et al., 2003). The promoter activity of the CAC PPRE was tested by transfecting HepG2 cells with pGL3 basic-LUC vector containing the $-334/+3$ bp region of the CAC gene (Iacobazzi et al., 2009). It was found that (i) fibrates upregulate CAC gene expression by binding to PPAR α , (ii) statins activate CAC gene expression by inhibiting the 3-hydroxy-3-methylglutaryl-CoA reductase and hence decreasing the levels of farnesyl and geranyl which control the Rho-signaling pathway (Martin et al., 2001), and (iii) fibrates and statins act synergistically. Moreover, CAC gene transcription is activated by forskolin, an activator of the PKA pathway, showing that this pathway also regulates CAC gene expression by a different mechanism – phosphorylation of PPAR α – from those exerted by statins and fibrates. Finally, retinoic acid activates PPRE when bound to the PPAR α -RXR α heterodimer (Fig. 6).

The role of FOXA and Sp1 sites in the regulation of CAC gene expression was investigated in HepG2, HEK293 and SK-N-SH cell lines (Convertini et al., 2011). Transfection of these cells with a construct containing the CAC promoter region from $-334/+3$ bp upstream of the LUC gene-coding sequence, showed that HepG2 cells exhibit a much higher gene reporter activity than HEK293 and SK-N-SH cells. Consistently HepG2 cells exhibited a higher level of CAC transcript and CAC protein than the other two cell types. By immunoprecipitation experiments it was demonstrated that both FOXA2 and Sp1 in HepG2 cells, and only Sp1 in HEK293 and SK-N-SH cells, are bound to the CAC proximal promoter (Convertini et al., 2011). In addition, strong evidence was provided that in HepG2 cells FOXA and Sp1 *cis*-elements of the CAC proximal promoter act as strong enhancers of CAC gene expression by interacting with their cognate transcriptional factors FOXA2 and Sp1. Both FOXA2 and Sp1 enable chromatin access to other transcriptional factors. However, Sp1 is an ubiquitously expressed transcription factor, while FOXA2 is a liver and pancreas specific transcriptional factor (Kaestner et al., 1994) essential for glucose and lipid

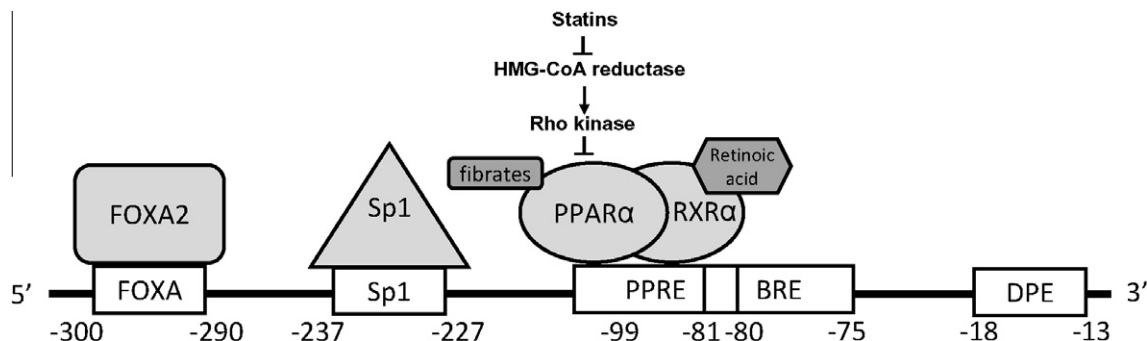


Fig. 6. Structural organization of the human CAC gene proximal promoter. The basal elements (BRE and DPE) and the positive responsive sequences (PPRE, FOXA and Sp1) as well as their cognate transcription factors are indicated. The effect of statins, through their inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and the Rho kinase pathway, and the interaction of fibrates and retinoic acid with PPAR α and RXR α , respectively, are also shown.

homeostasis (Wolfrum et al., 2004). In this respect it is interesting to note that, besides SLC25A20 encoding CAC, other genes encoding hepatic enzymes involved in metabolism during fasting and energy deprivation are transcriptionally regulated by FOXA2 (Wolfrum et al., 2004). Among these are enzymes of lipid catabolism, such as carnitine-palmitoyl-transferase 1, hydroxyacyl-CoA dehydrogenase, lipoprotein lipase, of ketogenesis, such as 3-hydroxy-3-methylglutaryl-CoA synthase 1, and of gluconeogenesis, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. It is likely that in liver Sp1 alone is not enough to activate the transcription of the CAC gene to a level sufficient to match the requirement of this tissue with respect to fatty acid metabolism. Thus FOXA2 is required for maximal activation of the CAC gene transcription. The presence of FOXA2 in liver could explain, at least in part, the differences in CAC levels of expression between liver and other tissues (Huizing et al., 1998a).

4. CAC in human pathology

4.1. Biochemical and clinical aspects of CAC deficiency

The biochemical, metabolic derangement and clinical aspects of CAC deficient patients are well characterized (Table 1). Biochemical derangement can be explained on the basis of the role of CAC in cell metabolism. Hypoglycemia is mainly due to excessive glucose utilization by peripheral tissues, especially heart and muscle, since the liver is unable to produce ketone bodies from fat during fasting. Rapid glycogen depletion and impaired gluconeogenesis also contribute to the mechanism of hypoglycemia. Thus, the inadequate rate of fatty acid oxidation and the inhibition of the ADP/ATP carrier by accumulation of long chain acyl-CoA result in insufficient production of ATP which is required for the first reactions of gluconeogenesis catalyzed by pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Moreover, reduced levels of acetyl-CoA cause an insufficient formation of citrate which stimulates 6-phosphofructose-1-kinase, the most important regulatory enzyme of glycolysis. Hypoketosis is a consequence of the reduced production of acetyl-CoA, the precursor of ketone bodies, 3-hydroxybutyric acid and acetoacetate. Hyperammonemia is due to increased catabolism of proteins, to diminished production of the allosteric activator, N-acetylglutamate, of urea cycle and to consequent liver failure. Dicarboxylic aciduria arises from activation of the ω -oxidation, which oxidizes the fatty acids accumulated due to fatty acid β -oxidation deficiency. Elevated transaminases and creatine kinase concentrations reflect the damage of heart, skeletal muscle and liver. Reduced free carnitine is a consequence of accumulation of acylcarnitines, which might inhibit the plasma membrane carnitine transporter (Stanley et al., 1992) leading to reduction of renal carnitine threshold and tissue carnitine deficiency.

As consequence of these biochemical abnormalities the clinical signs and symptoms reflect a combination of energy deficiency and endogenous toxicity. As expected, lack of energy affects fatty acid dependent tissues (heart, skeletal muscle and liver) leading to cardiomyopathies and liver dysfunction. Hypoketosis, hyperammonemia and severe hypoglycemia result in neurological dysfunction. Moreover, increased levels of acylcarnitines are toxic for muscle function and heart contributing to damage of heart and skeletal muscle. Other symptoms include vomiting, lethargy, hypotonia, weakness, respiratory distress and seizures.

CAC deficiency, first described by Stanley et al. (1992), is inherited as an autosomal recessive disorder. It presents two phenotypes: a severe phenotype, the most common, with onset in the neonatal period and a milder phenotype with onset in infancy or, less frequently, in childhood. Neonates with severe phenotypes deteriorate rapidly after birth following acute metabolic decompensation and intercurrent infections. The explanation of the high neonatal mortality rate is that β -oxidation is an essential metabolic pathway for energy supply in newborn infants. Infants with mild phenotype retain some residual CAC activity and respond to diet treatment. However, when both phenotypes are untreated, the severe hypoglycemic status may lead to brain damage, coma and death.

4.2. Diagnosis and therapy of CAC deficiency

Early recognition and treatment is crucial in CAC-deficient patients (Palmieri, 2008). Newborn screening programs leading to measurement of metabolites conjugated with carnitine, mainly acylcarnitines (up to 90% of total plasma carnitine), by mass spectrometry are usually used. Excessive excretion of dicarboxylic acids and paucity of fatty acid metabolites in urine

Table 1
Biochemical, serological and clinical features of CAC-deficient patients.

Biochemical and serological features	Clinical features
Hypoglycemia	Cardiomyopathy
Hyperammonemia	Arrhythmia
Hypoketosis	Liver dysfunctions
Dicarboxylic aciduria	Lethargy, convulsions
Increase of transaminases	Coma upon fasting
Increase of creatine kinase	Seizures
Increase of long chain acylcarnitines	Muscle weakness
Decrease of free carnitine	Respiratory distress

are also part of routine evaluation of patients with CAC deficiency. However, since dicarboxylic aciduria and the acylcarnitines profile observed in CAC-deficient patients are similar to those of most of fatty acid-oxidation disorders (Rubio-Gozalbo et al., 2004), identification of CAC-deficient patients requires determination of CAC activity and mutational analysis of CAC gene.

Several methods for determining CAC activity have been developed. The first procedure introduced by Pande et al. (1993) is carried out on fibroblasts or lymphocytes permeabilized with digitonin. This detergent forms complex with membrane cholesterol creating holes in the plasma membrane through which low molecular weight molecules can permeate, whereas mitochondrial integrity is not affected. The assay reaction contains malonate, [^{14}C]pyruvate, dichloroacetate and carnitine. Malonate inhibits Krebs cycle and blocks degradation of acetyl-CoA to CO_2 . [^{14}C]pyruvate and carnitine enter into mitochondria. [^{14}C]pyruvate is converted into [^{14}C]acetyl-CoA by pyruvate dehydrogenase, which is stimulated by dichloroacetate. Carnitine, through the action of carnitine acetyltransferase, is converted into [^{14}C]acetylcarnitine, which is exported outside mitochondria by CAC. The amount of this [^{14}C]acetylcarnitine reflects CAC activity (under the assumption that the other enzymes involved in the assay have normal activity). Later, IJlst et al. (2001) developed a procedure based on the ability of human CAC to complement the mutant yeast strain $\Delta\text{cit2}/\text{cac}$ which is unable to grow on oleate. A similar method based on the use of the *Aspergillus nidulans* ΔacuH mutant strain was developed by Pérez et al. (2003) after the identification of CAC (*acuH*) in *A. nidulans* (De Lucas et al., 1999). The latter two methods allow a functional analysis of the mutations found in patients affected by CAC deficiency. A more quantitative analysis of disease-causing CAC mutations can be obtained by measuring the activity of the recombinant CAC mutant proteins in reconstituted liposomes as described in Section 2.1. Differential diagnosis is based on the identification of a mutation in CAC gene and the finding that CPT1, CPT2 and the other enzymes of fatty acid β -oxidation are normal in terms of activity and gene DNA sequence. Furthermore, prenatal diagnosis is possible by assessing cultured fetal cells (trophoblasts or amniocytes) with an *in vitro* CAC assay or by using chorionic villus sampling for DNA analysis (Brivet et al., 2003). In conclusion, the combination of genetic test and activity determination allows a sure CAC deficiency diagnosis and provides exhaustive information on the effect of a defined mutation on the activity of CAC, which is useful for understanding both the CAC structure/function relationship and the correlation between the molecular defect and the phenotype.

Following diagnosis of CAC deficiency, an adequate treatment must be adopted. The first goal is to provide sufficient glucose to prevent adipose tissue lipolysis. Long-term treatment of CAC deficiency consists of fasting prevention with frequent meals, a diet rich in carbohydrates, low in lipids and supplemented with essential polyunsaturated fatty acids. In particular, 80% of available lipids should be represented by medium chain triglycerides (MCT). Since the C10 fatty acids mitochondrial transport depends on carnitine (Chalmers et al., 1997) and some MCT oil formulas contain high percentage of C10 fatty acids, low C10 fatty acid formulas should be preferred (Iacobazzi et al., 2004a; Rubio-Gozalbo et al., 2004). Use of carnitine is controversial; on one hand it exerts a protective effect of arrhythmias (Davini et al., 1992; Duan and Moffat, 1991) and on the other hand it causes an increase of acylcarnitines, responsible of arrhythmias. This discrepancy could be explained by the fact that in onset of cardiac problems are involved not only long chain acylcarnitines, but also increasing toxic lipid derivatives and free fatty acids. Conjugation with carnitine is a mechanism for removal and excretion of toxic metabolites. Long chain acylcarnitines, while capable of causing arrhythmias, might be less toxic than free fatty acids and other metabolites (Iacobazzi et al., 2004a). It should be emphasized that CAC-deficient patients are very vulnerable to each fasting period or illness that can lead to acute encephalopathy with risk of permanent neurological damage or death. This can be managed by avoiding fasting and maintaining high carbohydrate intake during illness and ammonia detoxification. However, in case of fever, vomiting or other illness administration of intravenous specific emergency protocol should be used. Recently, a pharmacological approach for CAC-deficient patients, who present a mild phenotype with some residual activity of CAC (Dionisi-Vici et al., 1995; Olpin et al., 1997; Morris et al., 1998; Lopriore et al., 2001; Iacobazzi et al., 2004b) has been proposed (Iacobazzi et al., 2009). As reported above, statins and fibrates upregulate CAC gene transcription via PPRE site both separately and synergistically (Fig. 6). It is believed that the above reported patients, possessing a defective CAC having a residual activity equal or greater than 10% of that displayed by wild-type CAC, can benefit from treatment with statins and/or fibrates acting via stimulation of CAC gene expression and consequent increase in total CAC activity. A similar approach has been successfully used for other disorders of fatty acid β -oxidation (Djouadi et al., 2003).

4.3. CAC gene mutations in patients affected by CAC deficiency

Since the first two mutations of SLC25A20 reported (Huizing et al., 1997, 1998b), a total of 35 mutations have been identified including the 8 novel mutations (Wang et al., 2011) that have not yet been registered in the Human Gene Mutation Database. All the naturally occurring point mutations, in-frame deletions and deletion/insertion mutations known to date are listed in Table 2. The other mutations are nine small deletions, two small insertions, three gross deletions and four splicing mutations. Unfortunately only a few of the mutations reported in Table 2 have been functionally analyzed. However, many of them concern residues thought to be important for the functioning of CAC. For example, D32N, R133W, P230R, D231H and Q238R regard amino acids belonging to the salt-bridge network that closes the carrier on the matrix side (matrix gate) and is therefore fundamental for the carrier opening/closing on the matrix side (Palmieri, 2008; Cappello et al., 2007); G81R and R178Q regard residues that have been proposed to bind the substrate (Robinson and Kunji, 2006; Giangregorio et al., 2010); C23R and G28C protrude into the carrier internal cavity which is part of the channel through which the substrate is exported into the mitochondrial matrix; and Glu-260 deletion concerns a highly conserved residue in mitochondrial

Table 2

Missense, nonsense, in-frame deletion and in-frame insertion/deletion mutations of CAC in patients affected by CAC deficiency.

Nationality	Onset	Residual enzyme activity	Mutation	Codon number	Amino acid change	Location in protein structure	Reference
Pakistan	Neonatal	2.7%	cCGT-TGT	28	Gly-Cys	Close to the Matrix gate	Costa et al. (2003)
Noth Africa	Neonatal	N.D.	gGAC-ACC	32	Asp-Asn	Matrix gate	Costa et al. (2003)
France	Neonatal	N.D.	aCGA-TGA	166	Arg-Term	Matrix loop	Costa et al. (1999)
Netherlands	Neonatal	N.D.	cGGG-AGG	81	Gly-Arg	Binding site	Ijlst et al. (1999)
Italy	Neonatal	N.D.	aCGG-TGG	133	Arg-Trp	Matrix gate	Costa et al. (2003)
North Africa	Neonatal	N.D.	CGA-CAA	178	Arg-Gln	Binding site	Costa et al. (2003)
Spain	Neonatal	N.D.	gCGA-TGA	178	Arg-Term	Binding site	Rubio-Gozalbo et al. (2003)
France	Neonatal	2%	CCA-CGA	230	Pro-Arg	Matrix gate	Costa et al. (2003)
Germany	Neonatal	<1%	aGAT-CAT	231	Asp-His	Matrix gate	Costa et al. (2003)
Saudi	Neonatal	<1%	CAG-CGG	238	Gln-Arg	Matrix gate	Al Aqeel et al. (2003)
Italy	Neonatal	<1%	GCG-GTG	281	Ala-Val	Two turns of helix above the binding site	Iacobazzi et al. (2004b)
Iran	5 months	N.D.	TGC-CGC	23	Cys-Arg	Two turns of helix above the matrix gate	Wang et al. (2011)
–	2 years old girl	N.D.	ACC-AAC	55	Thr-Asn	Matrix loop	Wang et al. (2011)
–	2 years old girl	N.D.	GCC-GTC	87	Ala-Val	Two turns of helix above the binding site	Wang et al. (2011)
Caucasian	Neonatal	N.D.	CGA-TGA	275	Arg-Term	Binding site	Wang et al. (2011)
Caucasian	2 months	N.D.	AAG del	260	Glu del	Matrix loop	Wang et al. (2011)
Spain	10 months	N.D.	c.159dupT; c.163delA	54–55	Gly/Thr del Trp/Ala ins	Matrix loop	Iacobazzi et al. (2004b)

Abbreviations: del, deletion; dup, duplication; ins, insertion; term, termination; N.D., not determined.

carriers which is located in the matrix loop connecting transmembrane α -helices H5 and H6 and forms a salt-bridge with a second positive residue of the signature motif in the 3D structure of the ADP/ATP carrier.

5. Future perspectives

Although significant progress has been made in the study of the biochemical properties of CAC and its role in eukaryotic intermediary metabolism, the molecular basis of the CAC-mediated transport mechanism is still far from being completely understood. Future resolution of the 3D structure of CAC, or other members of the mitochondrial carrier protein family, in different conformations (cytosolic-, matrix- and transition states) will be crucial for full comprehension of the catalytic mechanism of transport at the molecular level. Other areas that need to be further explored are the mechanisms of CAC activity regulation in different human tissues, hormonal and nutritional states. In particular the regulation of the CAC gene expression and the influence of oxidative stress on CAC activity in the cell should be thoroughly investigated. An important outcome of the molecular identification and characterization of CAC is the disclosure and the elucidation of CAC deficiency. The mutations in the CAC gene found in patients affected by this fatty acid oxidation disorder are distributed throughout the entire gene and seem to be pan-ethnic. However, the incidence of CAC deficiency in the various populations remains to be determined, as it is the case for many rare diseases. Because the present therapeutic approach has only partial and temporary success, an attractive and fully effective perspective in the treatment of CAC deficiency would be the desired forthcoming gene therapy.

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References

- Al Aqeel, A.I., Rashid, M.S., Ruiter, J.P., Ijlst, L., Wanders, R.J., 2003. A novel molecular defect of the carnitine acylcarnitine translocase gene in a Saudi patient. *Clin. Genet.* 64, 163–165.
- Brivet, M., Garcia-Cazorla, A., Lyonnet, S., Dumez, Y., Nassogne, M.C., Slama, A., Boutron, A., Touati, G., Legrand, A., Saudubray, J.M., 2003. Impaired mitochondrial pyruvate importation in a patient and a fetus at risk. *Mol. Genet. Metab.* 78, 186–192.
- Calvani, M., Reda, E., Arrigoni-Martelli, E., 2000. Regulation by carnitine of myocardial fatty acid and carbohydrate metabolism under normal and pathological conditions. *Basic Res. Cardiol.* 95, 75–83.
- Cappello, A.R., Miniero, D.V., Curcio, R., Ludovico, A., Daddabbo, L., Stipani, I., Robinson, A.J., Kunji, E.R., Palmieri, F., 2007. Functional and structural role of amino acid residues in the odd-numbered transmembrane α -helices of the bovine mitochondrial oxoglutarate carrier. *J. Mol. Biol.* 369, 400–412.

- Chalmers, R.A., Stanley, C.A., English, N., Wigglesworth, J.S., 1997. Mitochondrial carnitine–acylcarnitine translocase deficiency presenting as sudden neonatal death. *J. Pediatr.* 131, 220–225.
- Convertini, P., Infantino, V., Bisaccia, F., Palmieri, F., Iacobazzi, V., 2011. Role of FOXA and Sp1 in mitochondrial acylcarnitine carrier gene expression in different cell lines. *Biochem. Biophys. Res. Commun.* 404, 376–381.
- Costa, C., Costa, J.M., Nuoffer, J.M., Slama, A., Boutron, A., Saudubray, J.M., Legrand, A., Brivet, M., 1999. Identification of the molecular defect in a severe case of carnitine–acylcarnitine carrier deficiency. *J. Inher. Metab. Dis.* 22, 267–270.
- Costa, C., Costa, J.M., Slama, A., Boutron, A., Vequaud, C., Legrand, A., Brivet, M., 2003. Mutational spectrum and DNA-based prenatal diagnosis in carnitine–acylcarnitine translocase deficiency. *Mol. Genet. Metab.* 78, 68–73.
- Dalle-Donne, I., Rossi, R., Giustarini, D., Colombo, R., Milzani, A., 2007. S-glutathionylation in protein redox regulation. *Free Radic. Biol. Med.* 43, 883–898.
- Davini, P., Su, C.M., Ademes, V.R., Boem, A., 1992. Controlled study on L-carnitine therapeutic efficacy in post-infarction. *Drugs Exp. Clin. Res.* 18, 355–365.
- De Lucas, J.R., Domínguez, A.I., Valenciano, S., Turner, G., Laborda, F., 1999. The *acuH* gene of *Aspergillus nidulans*, required for growth on acetate and long-chain fatty acids, encodes a putative homologue of the mammalian carnitine/acyl-carnitine carrier. *Arch. Microbiol.* 171, 386–396.
- De Lucas, J., Indiveri, C., Tonazzi, A., Perez, P., Giangregorio, N., Iacobazzi, V., Palmieri, F., 2008. Functional characterization of residues within the carnitine/acylcarnitine translocase RX2PANAXF distinct motif. *Mol. Membr. Biol.* 25, 152–163.
- Dionisi-Vici, C., Garavaglia, B., Bartuli, A., Invernizzi, F., DiDonato, S., Sabetta, G., Kahler, S.G., Millington, D.S., 1995. Carnitine–acylcarnitine translocase deficiency: benign course without cardiac involvement. *Pediatr. Res.* 37, 147A.
- Djouadi, F., Bonnefont, J.P., Thuillier, L., Droin, V., Khadom, N., Munnich, A., Bastin, J., 2003. Correction of fatty acid oxidation in carnitine palmitoyl transferase 2-deficient cultured skin fibroblasts by bezafibrate. *Pediatr. Res.* 54, 446–451.
- Duan, J., Moffat, M.P., 1991. Protective effect of D,L-carnitine against arrhythmias induced by lysophosphatidylcholine or reperfusion. *Eur. J. Pharmacol.* 192, 355–363.
- Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239–247.
- Foster, D.W., 2004. The role of the carnitine system in human metabolism. *Ann. N. Y. Acad. Sci.* 1033, 1–16.
- Giangregorio, N., Tonazzi, A., Console, L., Indiveri, C., Palmieri, F., 2010. Site-directed mutagenesis of charged amino acids of the human mitochondrial carnitine/acylcarnitine carrier: insight into the molecular mechanism of transport. *Biochim. Biophys. Acta* 1797, 839–845.
- Giangregorio, N., Tonazzi, A., Indiveri, C., Palmieri, F., 2007. Conformation-dependent accessibility of Cys-136 and Cys-155 of the mitochondrial rat carnitine/acylcarnitine carrier to membrane-impermeable SH reagents. *Biochim. Biophys. Acta* 1767, 1331–1339.
- Huijgen, R., Vissers, M.N., Defesche, J.C., Lansberg, P.J., Kastelein, J.J., Hutten, B.A., 2008. Familial hypercholesterolemia: current treatment and advances in management. *Expert Rev. Cardiovasc. Ther.* 6, 567–581.
- Huizing, M., Iacobazzi, V., IJlst, L., Savelkoul, P., Ruitenbeek, W., van den Heuvel, L., Indiveri, C., Smeitink, J., Trijbels, F., Wanders, R., Palmieri, F., 1997. Cloning of the human carnitine–acylcarnitine carrier cDNA, and identification of the molecular defect in a patient. *Am. J. Hum. Genet.* 61, 1239–1245.
- Huizing, M., Ruitenbeek, W., van den Heuvel, L., Dolce, V., Iacobazzi, V., Smeitink, J., Palmieri, F., Trijbels, J., 1998a. Human mitochondrial transmembrane metabolite carriers: tissue distribution and its implication for mitochondrial disorders. *J. Bioenerg. Biomembr.* 30, 277–284.
- Huizing, M., Wendel, U., Ruitenbeek, W., Iacobazzi, V., IJlst, L., Veenhuizen, P., Savelkoul, P., van den Heuvel, L.P., Smeitink, J.A.M., Wanders, R.J.A., Trijbels, F.J.M., Palmieri, F., 1998b. Carnitine–acylcarnitine carrier deficiency: identification of the molecular defect in a patient. *J. Inher. Metab. Dis.* 21, 262–267.
- Iacobazzi, V., Convertini, P., Infantino, V., Scarcia, P., Todisco, S., Palmieri, F., 2009. Statins, fibrates and retinoic acid upregulate mitochondrial acylcarnitine carrier gene expression. *Biochem. Biophys. Res. Commun.* 388, 643–647.
- Iacobazzi, V., Naglieri, M., Stanley, C., Wanders, R., Palmieri, F., 1998. The structure and organization of the human carnitine/acylcarnitine translocase (CACT) gene. *Biochem. Biophys. Res. Commun.* 252, 770–774.
- Iacobazzi, V., Invernizzi, F., Baratta, S., Pons, R., Chung, W., Garavaglia, B., Dionisi-Vici, C., Ribes, A., Parini, R., Huertas, M.D., Roldan, S., Lauria, G., Palmieri, F., Taroni, F., 2004a. Molecular and functional analysis of SLC25A20 mutations causing carnitine–acylcarnitine translocase deficiency. *Hum. Mutat.* 24, 12–20.
- Iacobazzi, V., Pasquali, M., Singh, R., Matern, D., Rinaldo, P., Amat di San Filippo, C., Palmieri, F., Longo, N., 2004b. Response to therapy in carnitine/acylcarnitine translocase (CACT) deficiency due to a novel missense mutation. *Am. J. Med. Genet.* 126A, 150–155.
- Idell-Wenger, J.A., Grottyhann, L.W., Neely, J.R., 1978. Coenzyme A and carnitine distribution in normal and ischemic hearts. *J. Biol. Chem.* 253, 4310–4318.
- IJlst, L., Ruiters, J.P.N., Oostheim, W., Niezen-koning, K.E., Palmieri, F., Wanders, R.J.A., 1999. Identification of a missense mutation in a patient with lethal carnitine acyl-carnitine carrier deficiency. In: Quant, P.A., Eaton, S. (Eds.), *Current Views of Fatty Acid Oxidation and Ketogenesis: From Organelles to Point Mutations*. Kluwer Academic/Plenum Publishers, New York, pp. 347–351.
- IJlst, L., van Roermund, C., Iacobazzi, V., Oostheim, W., Ruiters, J., Williams, J., Palmieri, F., Wanders, R., 2001. Functional analysis of mutant human carnitine acylcarnitine translocases in yeast. *Biochem. Biophys. Res. Commun.* 280, 700–706.
- Indiveri, C., Giangregorio, N., Iacobazzi, V., Palmieri, F., 2002. Site-directed mutagenesis and chemical modification of the six native cysteine residues of the rat mitochondrial carnitine carrier: implications for the role of cysteine-136. *Biochemistry* 41, 8649–8656.
- Indiveri, C., Iacobazzi, V., Giangregorio, N., Palmieri, F., 1997. The mitochondrial carnitine carrier protein: cDNA cloning, primary structure and comparison with other mitochondrial transport proteins. *Biochem. J.* 321, 713–719.
- Indiveri, C., Iacobazzi, V., Giangregorio, N., Palmieri, F., 1998. Bacterial overexpression, purification, and reconstitution of the carnitine/acylcarnitine carrier from rat liver mitochondria. *Biochem. Biophys. Res. Commun.* 249, 589–594.
- Indiveri, C., Palmieri, F., 1989. Purification of the mitochondrial carnitine carrier by chromatography on hydroxyapatite and celite. *FEBS Lett.* 253, 217–220.
- Indiveri, C., Tonazzi, A., Giangregorio, N., Palmieri, F., 1995. Probing the active site of the reconstituted carnitine carrier from rat liver mitochondria with sulfhydryl reagents. A cysteine residue is localized in or near the substrate binding site. *Eur. J. Biochem.* 228, 271–278.
- Indiveri, C., Tonazzi, A., Palmieri, F., 1990. Identification and purification of the carnitine carrier from rat liver mitochondria. *Biochim. Biophys. Acta* 1020, 81–86.
- Indiveri, C., Tonazzi, A., Palmieri, F., 1991a. Characterization of the unidirectional transport of carnitine catalyzed by the reconstituted carnitine carrier from rat liver mitochondria. *Biochim. Biophys. Acta* 1069, 110–116.
- Indiveri, C., Tonazzi, A., Prezioso, G., Palmieri, F., 1991b. Kinetic characterization of the reconstituted carnitine carrier from rat liver mitochondria. *Biochim. Biophys. Acta* 1065, 231–238.
- Indiveri, C., Tonazzi, A., Dierks, T., Kramer, R., Palmieri, F., 1992. The mitochondrial carnitine carrier: characterization of SH-groups relevant for its transport function. *Biochim. Biophys. Acta* 1140, 53–58.
- Indiveri, C., Tonazzi, A., Palmieri, F., 1994. The reconstituted carnitine carrier from rat liver mitochondria: evidence for a transport mechanism different from that of the other mitochondrial translocators. *Biochim. Biophys. Acta* 1189, 65–73.
- Jones, D.P., 2006. Redefining oxidative stress. *Antioxid. Redox Signal.* 8, 1865–1879.
- Kaestner, K., Hiemisch, H., Luckow, B., Schütz, G., 1994. The HNF-3 gene family of transcription factors in mice: gene structure, cDNA sequence, and mRNA distribution. *Genomics* 20, 377–385.
- Kunji, E.R., Robinson, A.J., 2010. Coupling of proton and substrate translocation in the transport cycle of mitochondrial carriers. *Curr. Opin. Struct. Biol.* 20, 440–447.
- Lee, K., Kerner, J., Hoppel, C.L., 2011. Mitochondrial carnitine palmitoyltransferase 1a (CPT1a) is part of an outer membrane fatty acid transfer complex. *J. Biol. Chem.* 286, 25655–25662.
- Lopriore, E., Gemke, R.J., Verhoeven, N.M., Jakobs, C., Wanders, R.J., Roeleveld-Versteeg, A.B., 2001. Carnitine–acylcarnitine translocase deficiency: phenotype, residual enzyme activity and outcome. *Eur. J. Pediatr.* 160, 101–104.

- Martin, G., Duez, H., Blanquart, C., Berezowski, V., Poulain, P., Fruchart, J., Najib-Fruchart, J., Glineur, C., Staels, B., 2001. Statin-induced inhibition of the Rho-signaling pathway activates PPARalpha and induces HDL apoA-I. *J. Clin. Invest.* 107, 1423–1432.
- Morris, A.A., Olpin, S.E., Brivet, M., Turnbull, D.M., Jones, R.A., Leonard, J.V., 1998. A patient with carnitine–acylcarnitine translocase deficiency with a mild phenotype. *J. Pediatr.* 132, 514–516.
- Murthy, M.S.R., Pande, V.S., 1984. Mechanism of carnitine acylcarnitine translocase-catalyzed import of acylcarnitines into mitochondria. *J. Biol. Chem.* 259, 9082–9089.
- Olpin, S.E., Bonham, J.R., Downing, M., Manning, N.J., Pollitt, R.J., Sharrard, M.J., Tanner, M.S., 1997. Carnitine–acylcarnitine translocase deficiency – a mild phenotype. *J. Inher. Metab. Dis.* 20, 714–715.
- Palmieri, F., 2004. The mitochondrial transporter family (SLC25): physiological and pathological implications. In “The ABC of solute carriers”. *Pflügers Arch.* 447, 689–709.
- Palmieri, F., 2008. Diseases caused by defects of mitochondrial carriers: a review. *Biochim. Biophys. Acta* 1777, 564–578.
- Palmieri, F., Agrimi, G., Blanco, E., Castegna, A., Di Noia, M.A., Iacobazzi, V., Lasorsa, F.M., Marobbio, C.M.T., Palmieri, L., Scarcia, P., Todisco, S., Voza, A., Wlaker, J., 2006. Identification of mitochondrial carriers in *Saccharomyces cerevisiae* by transport assay of reconstituted recombinant proteins. *Biochim. Biophys. Acta* 1757, 1249–1262.
- Palmieri, F., Indiveri, C., Bisaccia, F., Iacobazzi, V., 1995. Mitochondrial metabolite carrier proteins: purification, reconstitution, and transport studies. *Methods Enzymol.* 260, 349–369.
- Palmieri, F., Indiveri, C., Bisaccia, F., Kramer, R., 1993. Functional properties of purified and reconstituted mitochondrial metabolite carriers. *J. Bioenerg. Biomembr.* 25, 525–535.
- Palmieri, F., Pierri, C.L., 2010a. Mitochondrial metabolite transport. *Essays Biochem.* 47, 37–52.
- Palmieri, F., Pierri, C.L., 2010b. Structure and function of mitochondrial carriers – role of the transmembrane helix P and G residues in the gating and transport mechanism. *FEBS Lett.* 584, 1931–1939.
- Palmieri, F., Pierri, C.L., De Grassi, A., Nunes-Nesi, A., Fernie, A.R., 2011. Evolution, structure and function of mitochondrial carriers: a review with new insights. *Plant J.* 66, 161–181.
- Palmieri, L., Lasorsa, F.M., Iacobazzi, V., Runswick, M.J., Palmieri, F., Walker, J.E., 1999. Identification of the mitochondrial carnitine carrier in *Saccharomyces cerevisiae*. *FEBS Lett.* 462, 472–476.
- Pande, S.V., Brivet, M., Slama, A., Demaugre, F., Aufrant, C., Saudubray, J.M., 1993. Carnitine–acylcarnitine translocase deficiency with severe hypoglycemia and auriculo ventricular block. Translocase assay in permeabilized fibroblasts. *J. Clin. Invest.* 91, 1247–1252.
- Paradies, G., Ruggiero, F.M., Petrosillo, G., Quagliariello, E., 1997. Alterations in carnitine–acylcarnitine translocase activity and in phospholipid composition in heart mitochondria from hypothyroid rats. *Biochim. Biophys. Acta* 1362, 193–200.
- Pebay-Peyroula, E., Dahout-Gonzalez, C., Kahn, R., Trezeguet, V., Lauquin, G.J., Brandolin, G., 2003. Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. *Nature* 426, 39–44.
- Pérez, P., Martínez, O., Romero, B., Olivas, I., Pedregosa, A., Palmieri, F., Laborda, F., Ramón De Lucas, J., 2003. Functional analysis of mutations in the human carnitine/acylcarnitine translocase in *Aspergillus nidulans*. *Fungal Genet. Biol.* 39, 211–220.
- Picault, N., Hodges, M., Palmieri, L., Palmieri, F., 2004. The growing family of mitochondrial carriers in *Arabidopsis*. *Trends Plant Sci.* 9, 138–146.
- Ramsay, R.R., Gandour, R.D., van der Leij, F.R., 2001. Molecular enzymology of carnitine transfer and transport. *Biochim. Biophys. Acta* 1546, 21–43.
- Reda, E., D'Iddio, S., Nicolai, R., Benatti, P., Calvani, M., 2003. The carnitine system and body composition. *Acta Diabetol.* 40 (Suppl. 1), S106–S113.
- Robinson, A.J., Kunji, E.R., 2006. Mitochondrial carriers in the cytoplasmic state have a common substrate binding site. *Proc. Natl. Acad. Sci. USA* 103, 2617–2622.
- Rubio-Gozalbo, M.E., Bakker, J.A., Waterham, H.R., Wanders, R.J., 2004. Carnitine–acylcarnitine translocase deficiency, clinical, biochemical and genetic aspects. *Mol. Aspects Med.* 25, 521–532.
- Rubio-Gozalbo, M.E., Vos, P., Forget, P.P., Van Der Meer, S.B., Wanders, R.J., Waterham, H.R., Bakker, J.A., 2003. Carnitine–acylcarnitine translocase deficiency: case report and review of the literature. *Acta Paediatr.* 92, 501–504.
- Rufer, A.C., Thoma, R., Hennig, M., 2009. Structural insight into function and regulation of carnitine palmitoyltransferase. *Cell. Mol. Life Sci.* 66, 2489–2501.
- Schoonjans, K., Staels, B., Auwerx, J., 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* 37, 907–925.
- Spadaro, D., Yun, B.W., Spoel, S.H., Chu, C., Wang, Y.Q., Loake, G.J., 2010. The redox switch: dynamic regulation of protein function by cysteine modifications. *Physiol. Plant.* 138, 360–371.
- Stanley, C.A., Hale, D.E., Berry, G.T., Deleuw, S., Boxer, J., Bonnefont, J.P., 1992. Deficiency of carnitine–acylcarnitine translocase in the inner mitochondrial membrane. *N. Engl. J. Med.* 327, 19–23.
- Tonazzi, A., Giangregorio, N., Indiveri, C., Palmieri, F., 2005. Identification by site-directed mutagenesis and chemical modification of three vicinal cysteine residues in rat mitochondrial carnitine/acylcarnitine transporter. *J. Biol. Chem.* 280, 19607–19612.
- Tonazzi, A., Giangregorio, N., Indiveri, C., Palmieri, F., 2009. Site-directed mutagenesis of the His residues of the rat mitochondrial carnitine/acylcarnitine carrier: implications for the role of His-29 in the transport pathway. *Biochim. Biophys. Acta* 1787, 1009–1015.
- Viggiano, L., Iacobazzi, V., Marzella, R., Cassano, C., Rocchi, M., Palmieri, F., 1997. Assignment of the carnitine/acylcarnitine translocase gene (CACT) to human chromosome band 3p21.31 by in situ hybridization. *Cytogenet. Cell Genet.* 79, 62–63.
- Wang, G.L., Wang, J., Douglas, G., Browning, M., Hahn, S., Ganesh, J., Cox, S., Aleck, K., Schmitt, E.S., Zhang, W., Wong, L.J., 2011. Expanded molecular features of carnitine acyl-carnitine translocase (CACT) deficiency by comprehensive molecular analysis. *Mol. Genet. Metab.* 103, 349–357.
- Wierzbicki, A.S., Mikhailidis, D.P., Wray, R., Schacter, M., Cramb, R., Simpson, W.G., Byrne, C.B., 2003. Statin–fibrate combination: therapy for hyperlipidemia: a review. *Curr. Med. Res. Opin.* 19, 155–168.
- Wolfgram, C., Asilmaz, E., Luca, E., Friedman, J., Stoffel, M., 2004. Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. *Nature* 432, 1027–1032.
- Zeth, K., Thein, M., 2010. Porins in prokaryotes and eukaryotes: common themes and variations. *Biochem. J.* 431, 13–22.