minireview

Molecular basis of mitochondrial fatty acid oxidation defects

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Abstract A dozen separate inherited disorders of mitochondrial fatty acid β-oxidation have been described in humans. This represents about half of the potential sites for genetic error that can affect this important pathway of energy metabolism. As the characterization of these disorders at the clinical and biochemical levels has progressed rapidly, so has the delineation of the molecular defects that underlie them. The most commonly recognized disorder of β-oxidation is medium-chain acyl-CoA dehydrogenase deficiency; a striking feature of this disorder is that there is a single point mutation that accounts for 90% of the variant alleles among patients with medium-chain acyl-CoA dehydrogenase deficiency. Molecular defects of other enzymes in the pathway have been identified, and it seems likely that a complete description of these defects at the molecular level is a realistic goal. In basic biological terms, such studies will lead to a better understanding of the genetic control exerted on this pathway. In clinical terms, they will lead to improved understanding of the molecular pathophysiology of these diseases and may well provide the necessary techniques to proceed with the screening of these disorders.—**Coates, P. M., and K. Tanaka.** Molecular basis of mitochondrial fatty acid oxidation defects. *J. Lipid Res.* 1992. **33:** 1099–1110.

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FATTY ACID β-OXIDATION

β-Oxidation systems: organelles and enzymes

The β-oxidation of fatty acids in eukaryotic organisms takes place in two distinct subcellular organelles, mitochondria and peroxisomes (1). The two β-oxidation systems are similar, but under entirely separate genetic control. Mitochondrial β-oxidation is linked to the respiratory chain, while peroxisomal β-oxidation is not. The mitochondrial import of fatty acids is carnitine-mediated, while this is not so for peroxisomes. In the following section, we will describe mitochondrial β-oxidation before discussing the inherited defects of this pathway.

Overall, the mitochondrial β-oxidation of a long-chain fatty acid (14 carbons or greater) requires the concerted action of 15–20 separate steps that include: cellular uptake of fatty acids and their activation to form acyl-CoA esters; the carnitine cycle, comprising the cellular uptake of carnitine and the carnitine-mediated translocation of acyl-CoA esters across the mitochondrial membrane; spirals of β-oxidation; enzymes of unsaturated fatty acid β-oxidation; and transfer of electrons to the electron transport chain. The interrelationships of these processes are presented schematically in **Fig. 1**.

A long-chain fatty acid is activated by acyl-CoA synthetase to form an acyl-CoA. This cannot readily traverse the mitochondrial membrane, but instead is transesterified to an acylcarnitine by carnitine palmitoyltransferase I (CPT I). Carnitine/acylcarnitine translocase (TRANS) mediates its entry into the mitochondrial matrix, where CPT II converts it back to the acyl-CoA. It is the acyl-CoA that enters the β-oxidation spiral, in which four successive reactions are mediated by acyl-CoA dehydrogenase, 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase. The products of each turn of the spiral are electrons, acetyl-CoA, and an acyl-CoA now 2 carbons shorter. An acyl-CoA can recycle through β-oxidation as many times as it can yield acetyl-CoA fragments.

Abbreviations: CPT I and CPT II, carnitine palmitoyltransferases I and II; ETF, electron transfer flavoprotein; ETF:QO, ETF:ubiquinone oxidoreductase; LCAD, MCAD, SCAD, and VLCAD, long-chain, medium-chain, short-chain, and very-long-chain acyl-CoA dehydrogenases; LCHAD and SCHAD, long-chain and short-chain 3-hydroxyacyl-CoA dehydrogenases; TRANS, carnitine/acylcarnitine translocase.

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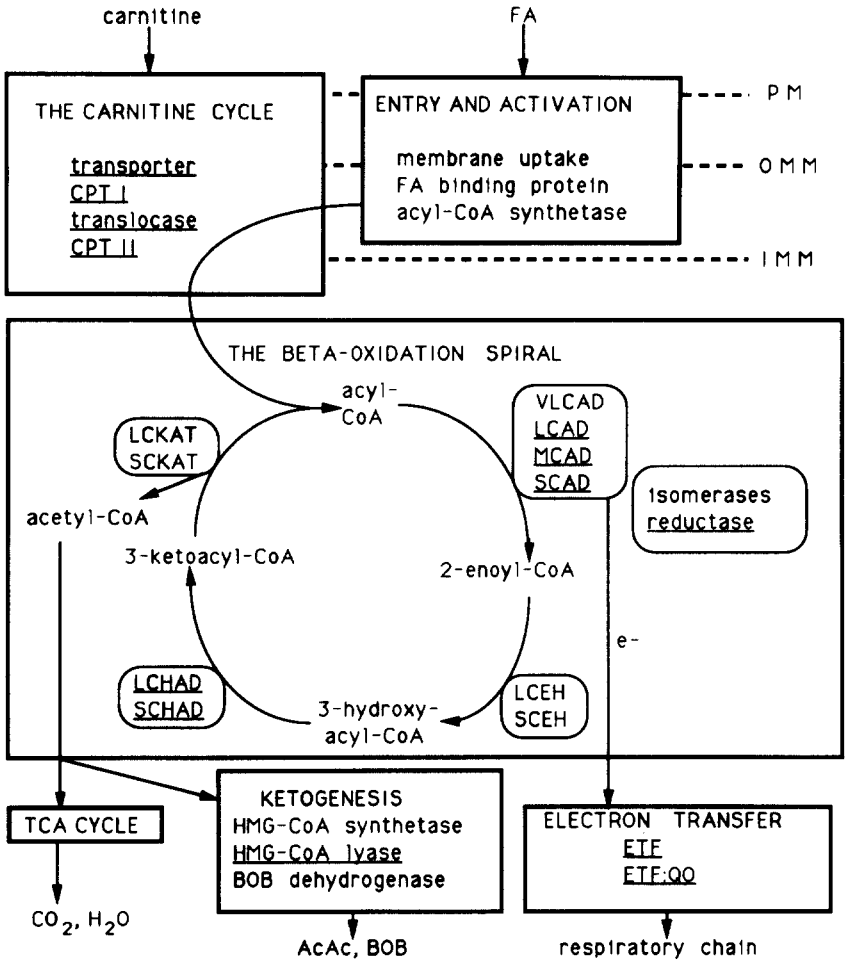

Fig. 1. Integration of mitochondrial fatty acid β-oxidation with other cellular pathways. Underlined enzymes depict sites of genetic defects identified in man. Abbreviations used: AcAc, acetoacetate; BOB, β-hydroxybutyrate; CPT, carnitine palmitoyltransferase; e^-, electron; ETF, electron transfer flavoprotein; ETF:QO, ETF:ubiquinone oxidoreductase; FA, fatty acid; HMG, 3-hydroxy-3-methylglutaryl; IMM, inner mitochondrial membrane; LCAD, MCAD, SCAD, VLCAD, long-chain, medium-chain, short-chain, and very-long-chain acyl-CoA dehydrogenases; LCEH, SCEH, long-chain and short-chain 2-enoyl-CoA hydratases; LCHAD, SCHAD, long-chain and short-chain 3-hydroxyacyl-CoA dehydrogenases; LCKAT, SCKAT, long-chain and short-chain 3-ketoacyl-CoA thiolases; OMM, outer mitochondrial membrane; PM, plasma membrane; TRANS, carnitine/acylcarnitine translocase.

With each turn of the spiral, as the acyl-CoA becomes shorter, it encounters enzymes with differing substrate specificities. The best example of this is the series of chain-length-specific acyl-CoA dehydrogenases. Long-chain acyl-CoA dehydrogenase (LCAD) mediates the reaction for acyl-CoA compounds from 8 carbons to 18 carbons; medium-chain acyl-CoA dehydrogenase (MCAD) from 4 to 12 carbons; and short-chain acyl-CoA dehydrogenase (SCAD) from 4 to 6 carbons (2). Recently, a new acyl-CoA dehydrogenase (very-long-chain acyl-CoA dehydrogenase, VLCAD) with activity towards acyl-CoA esters greater than 16 carbons in length has been identified in rat liver mitochondria (3). Long-chain fatty acids with double bonds, such as linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$), require additional enzymes—Δ^3, Δ^2-enoyl-CoA isomerases and 2,4-dienoyl-CoA reductase—for their complete oxidation (1, 4).

In the liver, the metabolic end-product of each cycle of β-oxidation, acetyl-CoA, is primarily converted to ketone bodies via the 3-hydroxy-3-methylglutaryl (HMG)-CoA pathway, while in other tissues, acetyl-CoA is completely oxidized via the citric acid cycle. Electrons from the acyl-CoA dehydrogenase-mediated reactions are transferred to electron transfer flavoprotein (ETF); transfer is from the flavin of the primary dehydrogenase (LCAD, MCAD, SCAD) to the flavin of ETF, to the flavin and then the iron–sulfur cluster of ETF:ubiquinone oxidoreductase (ETF:QO), and finally to the ubiquinone pool (5).

β-Oxidation enzymes: genes, biosynthesis, and mitochondrial processing

All of the enzymes involved in β-oxidation are listed in **Table 1**, along with some of their molecular properties. All are encoded in the nucleus, and cDNAs of most of them have been cloned and sequenced, providing data that are essential for the study of the molecular basis of disorders of fatty acid oxidation.

Due to the peculiar elongated nature of fatty acids, β-oxidation consists of repeated cycles of the same four sequential reactions, with each cycle handling substrates of different chain length. The three acyl-CoA dehydrogenases that catalyze the first reaction (LCAD, MCAD, and SCAD) have been isolated from various mammalian sources and their enzymatic properties have been well defined (2). cDNAs for the three acyl-CoA dehydrogenases in both rats and humans have been cloned and sequenced (9–14). Within the same species, these enzymes share approximately 30% sequence identity, indicating that they evolved from a common ancestral gene, but they do not cross-react immunologically. This is in contrast to the same enzyme from different mammalian species, which share 87–90% sequence identity and which generally cross-react immunologically. The information learned about the molecular relationships among the acyl-CoA dehydrogenases will undoubtedly be useful in understanding these relationships among the other enzymes of β-oxidation that exhibit overlapping chain-length specificity.

In general, nuclear gene products that are destined for mitochondrial import are synthesized on cytoplasmic

TABLE 1. Enzymes involved in mitochondrial fatty acid oxidation

Enzyme (Abbreviation)	Native Structure (Source)	Mature Subunit(s), kDa (Ref.)	cDNA Cloned and Sequenced (Ref.)
Fatty acid activation			
Acyl-CoA synthetase	?	?	No
Carnitine cycle			
Plasma membrane carnitine transporter	?	?	No
Carnitine palmitoyltransferase I (CPT I)	(rat muscle)	?	No
	(rat liver)	96 (6)	No
Carnitine/acylcarnitine translocase (TRANS)	(rat liver)	32.5 (7)	No
Carnitine palmitoyltransferase II (CPT II)	(human)	71 (6)	Yes (8)
Mitochondrial β-oxidation spiral			
Very-long-chain acyl-CoA dehydrogenase (VLCAD)	homodimer (rat liver)	71(3)	No
Long-chain acyl-CoA dehydrogenase (LCAD)	homotetramer (human and rat)	45 (2)	Yes (9,10)
Medium-chain acyl-CoA dehydrogenase (MCAD)	homotetramer (human and rat)	44 (2, 11)	Yes (12, 13)
Short-chain acyl-CoA dehydrogenase (SCAD)	homotetramer (human and rat)	41 (2, 11)	Yes (10, 14)
Trifunctional protein	heterooctamer (rat liver)	α = 80 (15) β = 50 (15)	No No
1. Long-chain 2-enoyl-CoA hydratase[a]			
2. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD)[a]			
3. Long-chain 3-ketoacyl-CoA thiolase[a]			
Short-chain 2-enoyl-CoA hydratase	homohexamer (rat)	26 (16)	Yes (17)
Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD)	homodimer (rat)	31 (16)	Yes (16)
Short-chain 3-ketoacyl-CoA thiolase	homotetramer (human)	42 (16)	Yes (18)
Enzymes of unsaturated β-oxidation			
Long-chain Δ^3,Δ^2-enoyl-CoA isomerase	200,000 kDa (4) (rat)	?	No
Short-chain Δ^3,Δ^2-enoyl-CoA isomerase	homodimer (rat)	29 (19)	Yes (19)
2,4-Dienoyl-CoA reductase	homotetramer (beef)	32 (20)	No
Electron transfer			
Electron transfer flavoprotein (ETF)	heterodimer (human)	α = 32 (21) β = 27 (21)	Yes (22) Yes (23)
ETF:ubiquinone oxidoreductase (ETF:QO)	monomer (human)	68 (24)	Yes (25)

[a]Activities associated with the trifunctional protein.

ribosomes, usually as precursor proteins with N-terminal extensions that guide them to the mitochondrial membrane. These so-called leader peptides are a few to several kDa in size; they are generally rich in basic amino acids and poor in acidic amino acids (26). Receptor-mediated, energy-dependent uptake of the precursor polypeptides is followed by proteolytic cleavage of their leader peptides (when present), yielding mature polypeptides. These are then assembled into fully active enzymes. The enzymes of β-oxidation are synthesized according to this pattern (16, 27), but there are some exceptions [e.g., the β-subunit of ETF (21) and 3-ketoacyl-CoA thiolase (16)].

INHERITED DEFECTS OF MITOCHONDRIAL FATTY ACID OXIDATION

Given the number of individual enzymatic steps involved in fatty acid β-oxidation and the complexity of enzyme processing, it is not surprising that many genetic defects of the pathway have been identified in humans. It is, however, remarkable that all of the inherited abnormalities of this pathway have been so recently elucidated. The first documented disorder affecting the mitochondrial β-oxidation of long-chain fatty acids in humans, muscle CPT deficiency, was described in 1973 (28). Since that time, a total of 12 inherited β-oxidation disorders have been described (24, 28–41). These are listed in **Table 2**, by the year of their first publication. Using MCAD deficiency as an example, we will briefly describe the features of mitochondrial β-oxidation disorders before providing details of specific molecular defects in the pathway.

TABLE 2. Defects of mitochondrial fatty acid oxidation in man

Fatty Acid Oxidation Defect (Reference)	Year of First Description
Muscular CPT[a] (adult-onset CPT II) deficiency[b] (28)	1973
Hepatic CPT (CPT I) deficiency (29)	1980
MCAD deficiency (30–32)	1982[c]
LCAD deficiency (33)	1985
ETF deficiency (24)	1985[c]
ETF:QO deficiency (24)	1985[c]
SCAD deficiency (34)	1987[c]
LCHAD deficiency (35)[d]	1988
Carnitine transport defect (36, 37)	1988
2,4-Dienoyl-CoA reductase deficiency (38)	1990
Hepatomuscular CPT (infantile CPT II) deficiency[b] (39)	1991[c]
Carnitine/acylcarnitine translocase deficiency (40)	1991
SCHAD deficiency (41)	1991

[a]Abbreviations: see Table 1.
[b]Probably mild and severe defects of the same gene.
[c]Defects in some patients have been characterized at the molecular level; see text for details.
[d]May represent deficiency of a trifunctional protein with 2-enoyl-CoA hydratase, LCHAD, and 3-ketoacyl-CoA thiolase activities (15, 95).

MCAD deficiency

Clinical and biochemical features. MCAD deficiency was identified independently in 1982–1983 by three groups of investigators (30–32). This disorder is the most commonly recognized β-oxidation defect in humans. It is the hallmark disorder of the pathway, as patients exhibit many of the signs and symptoms that we have come to recognize in this group of disorders (42). Episodes of fasting-induced lethargy, vomiting, and coma, beginning within the first 2 years of life, are the features most often recognized among patients with MCAD deficiency. Sometimes there is a prodromal viral illness, which has raised the possibility of Reye syndrome. Occasionally, patients have died suddenly and without prior symptoms, suggesting sudden infant death syndrome. Laboratory findings include: hypoglycemia; an inappropriately low ketone body response for the degree of fasting stress; mild acidosis; moderately increased blood levels of transaminases and ammonia; an organic aciduria characterized by increased excretion of the medium-chain dicarboxylic acids, adipic (C_6), suberic (C_8), and sebacic (C_{10}) acids, in the face of low β-hydroxybutyrate excretion; and low plasma and tissue carnitine levels. Hepatic neutral lipid storage is evident during acute episodes, which disappears upon recovery. As experience has been gained with the evaluation of patients with other fatty acid oxidation defects shown in Table 2, it has become clear that these features are sufficient to suggest a failure of mitochondrial β-oxidation, but not necessarily sufficient to make the specific diagnosis of MCAD deficiency. For example, there are no obvious signs of muscle or cardiac involvement (e.g., skeletal muscle weakness, myoglobinuria, or cardiomyopathy) in MCAD deficiency; these features can be prominent in patients with other fatty acid oxidation disorders (42).

Indirect lines of evidence have previously suggested that MCAD deficiency is a common disorder. Over the last few years, at least 200 patients have been identified world-wide. The ethnic distribution is striking, since virtually all of the reported patients are Caucasians (43–48). Several estimates of the frequency of this disorder have been made using indirect methods; these have ranged from 1:10,000 to 1:25,000. These incidence figures can now be better refined, using molecular methods to detect individual mutations in readily available samples, such as neonatal blood screening cards (49–51).

Diagnosis. It has been generally true that prompt diagnosis and treatment of patients with MCAD deficiency results in good long-term outlook. On the other hand, a failure to recognize the disease, or to institute appropriate dietary therapy, has meant that some children with MCAD deficiency have died suddenly and unexpectedly after a single episode or following a subsequent episode. The cornerstone of long-term management of patients

with MCAD deficiency is the avoidance of fasting and the maintenance of adequate caloric intake (42).

With the development of sophisticated techniques for the characterization of fatty acid intermediates and their derivatives in biological fluids, it is possible to detect metabolites that are uniquely present in the urine and blood of patients with MCAD deficiency. Acyl groups that accumulate as a result of incomplete β-oxidation can undergo other metabolic conversions within cells, to compounds such as dicarboxylic acids, acylglycines (52), and acylcarnitines (42, 53), which are more soluble than the corresponding acyl-CoAs and can be rapidly excreted in the urine. Other metabolites have also been identified which appear to be unique to MCAD deficiency, including plasma *cis*-4-decenoic acid (54). The specific enzyme deficiency has been diagnosed in several ways in a number of available tissues, including fibroblasts, leukocytes, and solid tissues such as liver and muscle (30–32, 55, 56). Patients generally have profoundly reduced MCAD activity, while their parents have intermediate levels, consistent with autosomal inheritance (55).

Molecular pathogenesis. The gene encoding human MCAD is located on chromosome 1, band p31 (57), and spans 44 kb of DNA in 12 exons (58). Both human and rat MCAD cDNAs encode a precursor subunit of 421 amino acids (12, 13), and there is a high degree of homology (87.2%) between the human and rat MCAD sequences. The rat precursor MCAD subunit consists of a 25-amino acid leader peptide and a 396-amino acid mature subunit (13). Although the N-terminus of mature human MCAD is unknown at present, it is presumably identical to that of the rat sequence. As is the case for all of the acyl-CoA dehydrogenases (with the exception of the recently described VLCAD, ref. 3), the fully active MCAD enzyme is a homotetramer containing 1 mol of FAD per subunit (2).

Within the last 2 years, several groups have identified a single, highly prevalent mutation within the coding region of MCAD cDNA among patients with documented MCAD deficiency (43–48, 59). This mutation, an A→ G transition at base 985 of MCAD cDNA (A_{985}→G), alters the amino acid sequence in an α-helical region in the carboxy-half of the MCAD subunit: lysine is replaced with glutamic acid at residue 329 of the precursor MCAD subunit (hence, the designation by some as K329E), which corresponds to residue 304 of the mature intramitochondrial subunit.

In a series of 172 European and US patients with confirmed MCAD deficiency (59), 138 (80.2%) were found to be homozygous for A_{985}→G; a further 30 patients (17.4%) carried this allele in heterozygous combination with another variant MCAD; and 4 patients (2.3%) did not have the A_{985}→G transition on either allele. It therefore accounts for 90% of the variant alleles characterized in these patients (**Table 3**).

Even before the discovery of this highly prevalent mutation, studies aimed at understanding the molecular basis of MCAD deficiency were undertaken by Ikeda et al. (60). Nascent MCAD in cultured fibroblasts was pulse-labeled with [^{35}S]methionine to examine the initial steps in biosynthesis of the precursor MCAD subunit and its proteolytic processing to a mature subunit within the mitochondrial matrix. The results demonstrated that these cells uniformly produced a variant MCAD subunit of normal size. All but one of the patients in this series were later found to be homozygous for the A_{985}→G mutation (44). Recently, we (61) and others (62, 63) have shown, by Western blot analysis of MCAD in fibroblasts, that all patients with MCAD deficiency have no, or very little, immunoreactive MCAD. Thus, despite normal translation and immediate posttranslational processing of MCAD in MCAD-deficient fibroblasts, virtually no variant MCAD protein is detectable in the steady state, suggesting that the variant MCAD protein within mitochondria in these cells is unstable. This is supported by additional metabolic labeling experiments (61), in which cells were incubated with [^{35}S]methionine for 1 h and then with unlabeled methionine for periods up to 24 h. These experiments

TABLE 3. Frequency of variant alleles at the MCAD locus (n = 344)

Mutation	Amino Acid Affected	Number of Alleles	Percentage
1. A_{985}→G	Lys_{329}→Glu	306	88.9%
2. 13 bp repeat 999–1012	(premature termination)	3	
3. 4 bp deletion 1100–1103	(premature termination)	2	
4. G_{799}→A	Gly_{267}→Arg	2	3.6%
5. C_{157}→T	Arg_{53}→Cys	1	
6. G_{447}→A	Met_{149}→Ile	1	
7. T_{730}→C	Cys_{244}→Arg	1	
8. T_{1124}→C	Ile_{375}→Thr	1	
Unidentified alleles		27	7.8%

Adapted from ref. 59.

showed that variant MCAD protein, in contrast to normal MCAD, disappears almost completely after 24 h of incubation with unlabeled methionine. These data are at variance with the earlier data of Kelly et al. (45) and Gregersen et al. (46), whose Western blot data demonstrated that fibroblasts from a few other patients homozygous for this mutation had immunodetectable MCAD. There is as yet no obvious explanation for the discrepancy between these two sets of findings.

Several mechanisms are possible to explain the apparent lability of the variant MCAD product of the $A_{985}\rightarrow G$ mutant allele. These include: inherent instability of the variant subunit; inhibition of tetramer formation; and disruption of normal tetramer structure. Computer analysis has predicted that the lysine-304 to glutamic acid substitution would not cause drastic changes in MCAD secondary structure (44). Residue 304 is located in a domain that is not involved in FAD binding or in substrate binding (64), but instead acts as the interface in the tetramer structure (65). It is an attractive hypothesis that substitution of a basic residue in this region with an acidic one may hinder tetramer formation or disrupt normal tetramer structure.

Restriction fragment polymorphism (RFLP) analysis by us (48) and by others (66) of the MCAD gene from numerous MCAD-deficient patients homozygous for the $A_{985}\rightarrow G$ mutant allele revealed that all of them belong to a single haplotype. This haplotype is one of four known types and accounts for only 35% of the general population (67, 68). Our own survey of the ethnic background of MCAD-deficient patients with this allele demonstrated that all were Caucasians, and 90% of them were of Northern European origin. The number of patients of English ancestry was by far the highest, followed by those from Germany. The tight linkage of this mutation with a single haplotype and its particularly high incidence in one geographic region is called the "founder effect"; it suggests that the $A_{985}\rightarrow G$ transition may be traced to a single progenitor, probably in an ancient Anglo-Saxon tribe.

Several other mutations have also been identified in MCAD cDNA from a few patients (Table 3), although none of these mutations has been found in more than 1% of variant alleles. These include five base transitions (48, 59) each of which causes an amino acid substitution, a 13bp repeat insert (44), and a 4bp deletion (69, 70). Each of the last two causes a frame shift leading to a premature termination codon, which results in a truncated protein.

LCAD deficiency

This disorder, first described in 1985 (33), is generally characterized by a more severe and earlier clinical presentation than is MCAD deficiency, affecting not just the liver, but also heart and skeletal muscle. This is likely due to the fact that long-chain fatty acid oxidation defects, as opposed to those affecting only medium-chain fatty acid oxidation, have widespread effects, and is consistent with the more severe presentation seen in patients with other defects of long-chain fatty acid oxidation (e.g., LCHAD deficiency and TRANS deficiency). Fewer than 20 patients have been identified with LCAD deficiency. The only specific diagnostic test is demonstration of LCAD deficiency in fibroblasts or other tissues (33). Parents have intermediate levels of LCAD activity, consistent with autosomal inheritance.

The human LCAD gene has been localized to chromosome 2, bands q34–q35, and its cDNA has been cloned and sequenced (9). The precursor LCAD subunit contains 430 amino acids (a 30-amino acid leader peptide and a 400-amino acid mature subunit). There is as yet no published report of any molecular defect(s) underlying LCAD deficiency. Western blot analysis shows that cells from all LCAD-deficient patients studied have immunoreactive LCAD (71).

SCAD deficiency

This rare disorder has been identified in only three patients (34, 72) with very different clinical presentations (neonatal hyperammonemic coma in one; intermittent episodes of metabolic acidosis in one; chronic progressive lipid storage myopathy in one). The enzyme deficiency was demonstrated in fibroblasts of all three patients. Another patient has been described (73) in whom the SCAD deficiency was isolated to skeletal muscle. A mouse model of SCAD deficiency has been described (74), something that does not yet exist for any of the other defects of fatty acid oxidation.

The human SCAD gene is on chromosome 12, q22–qter (75), and its cDNA has been cloned and sequenced (14). It encodes a 412-amino acid precursor, containing a 24-amino acid leader peptide and a 388-amino acid mature subunit. Two distinct mutations have been described in one of the three patients (76); one allele contains a $C_{136}\rightarrow T$ transition leading to an arginine-22 to tryptophan substitution, while the other allele contains a $C_{319}\rightarrow T$ transition leading to an arginine-83 to cysteine substitution. Variant SCAD in this patient's cells resembles variant MCAD due to the common $A_{985}\rightarrow G$ mutation, because it is unstable within the mitochondrial matrix (77).

CPT defects

CPT I deficiency. This disorder has been identified in less than a dozen patients (29, 78). It is characterized by episodic hypoketotic hypoglycemia beginning in early life, and hence has been referred to as hepatic CPT deficiency. It is rarely associated with muscular findings (79). It is distinguished from the other disorders of fatty acid oxidation by the fact that patients have high, rather than low, plasma carnitine levels; furthermore, they excrete little or no dicarboxylic acids in urine. Among patients with this

disorder, CPT I activity is very low in liver, fibroblasts, and leukocytes, but it is normal in muscle, suggesting that there are tissue-specific isozymes of CPT I (80). Parents' fibroblasts have intermediate levels of CPT I activity, consistent with autosomal inheritance. There is no information about the molecular basis of CPT I deficiency.

CPT II deficiency. There are several clinical presentations of CPT deficiency. The most common, as well as the most benign, is muscular CPT deficiency, described originally in 1973 (28). It is now known to be a deficiency of the inner mitochondrial CPT II activity. Patients with this defect generally present in adulthood with episodic muscle weakness prompted by prolonged exercise, cold, or fever. In spite of the primarily muscular presentation, their enzyme defect is not restricted to muscle, but can also be demonstrated in other tissues. More recently, a severe infantile hepatomuscular form of CPT II deficiency has been recognized, in which symptoms of hypoketotic hypoglycemia and cardiomyopathy suggest pathologic involvement of other tissues (39).

The gene for human CPT II is on chromosome 1 (8). In a patient with the severe infantile form of CPT II deficiency, a single C→T transition was found in homozygous form (81), which results in an arginine→cysteine substitution. This mutation apparently has no effect on the synthesis or processing of CPT II, which was present in fibroblasts in normal amounts and of normal size. Transfection of variant CPT II cDNA into cos-1 cells demonstrated its association with significantly reduced CPT II activity. In another patient with severe CPT II deficiency, decreased biosynthesis of protein has been observed (82), suggesting that there are likely to be other mutations underlying this disorder.

Glutaric acidemia Type II (GA II)

The clinical presentation of GA II is variable (83). In its most severe form, GA II presents in infancy with metabolic acidosis, hypoglycemia, and a "sweaty feet" odor, along with polycystic and dysplastic kidneys. Some patients have a milder disease, which may be characterized by recurrent episodes of fasting lethargy or slowly progressive myopathy. It is caused by genetic defects of either ETF or ETF:QO (24, 84). These two flavoproteins are required for the successive transfer of electrons from the primary acyl-CoA dehydrogenases in both fatty acid oxidation and branched-chain amino acid oxidation to the mitochondrial electron transport chain, hence the term multiple acyl-CoA dehydrogenation disorders which often has been used to describe this group of diseases (85).

ETF deficiency. ETF is a dimeric protein whose subunits are referred to as α-ETF and β-ETF. Each dimer contains 1 equivalent of FAD, presumably in the β-subunit (86). α-ETF is encoded by a gene on chromosome 15 (75), and is synthesized as a 35 kDa precursor that is imported into the mitochondrial matrix and processed to its mature 32 kDa form by proteolytic cleavage (21). By contrast, the 27 kDa β-ETF subunit, encoded on chromosome 19 (23), is imported intact and undergoes no further processing. cDNAs for both subunits have been cloned and sequenced (22, 23), and defects of both α- and β-subunits have been identified. Some of these affect the biosynthesis and mitochondrial import of the precursors and hence are characterized by an unstable mature protein (87–89).

ETF:QO deficiency. ETF:QO is an integral membrane flavoprotein containing 1 equivalent of FAD and an iron-sulfur (4Fe-4S) cluster (5). Human ETF:QO cDNA has been cloned and sequenced (25); it encodes a protein of 617 amino acids, comprising a leader peptide and a mature polypeptide, in which functional domains for FAD binding (amino terminus) and iron-sulfur cluster binding (carboxy terminus) can be identified. Some patients with ETF:QO deficiency have immunodetectable ETF:QO protein, while others do not (84). Among patients without detectable antigen, three different mutations have been found. Two are single base deletions and the third is a 201-base deletion which may be due to exon skipping. All three of these mutations change or delete the iron-sulfur cluster (25).

Other fatty acid oxidation defects

LCHAD deficiency. This disorder has been described in about 20 patients world-wide (35, 90–94) and is characterized frequently by severe clinical illness with hypoketotic hypoglycemia, muscle weakness, cardiomyopathy, hepatic dysfunction, and rarely by progressive peripheral neuropathy and pigmented retinopathy. The urinary excretion of long-chain 3-hydroxydicarboxylic acids is often the clue to diagnosis of LCHAD deficiency. The enzyme defect is expressed in all tissues examined; parents have intermediate levels of LCHAD activity. Recent data (15, 95) suggest that this enzyme activity in rat and human liver mitochondria may reside in a trifunctional protein (Table 1) with long-chain 2-enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activities. This is supported by the data from a few patients with LCHAD deficiency who also have reduced levels of these other two enzyme activities (91, 94). In most patients, however, the long-chain enoyl-CoA hydratase and ketoacyl-CoA thiolase activities have not been measured. There is no information yet available on molecular defects of this system.

Carnitine transport defect. This disorder is a true primary systemic carnitine deficiency for several reasons: it results in extremely low plasma and tissue carnitine levels; it is not secondary to an intramitochondrial defect of organic acid oxidation; and patients with this disorder respond dramatically to carnitine therapy. There have been reports on 20 patients (36, 37, 96, 97) with this defect, which results from a failure of plasma membrane carnitine uptake through a high-affinity system present in muscle, heart, kidney, and fibroblasts. The liver apparently

does not express this high-affinity carnitine transporter. The properties of this transport system are as yet poorly understood. Parents' cells have intermediate levels of transport activity, suggesting autosomal inheritance of the defect. No information is yet available about the molecular aspects of this defect.

TRANS deficiency. Recently, Stanley, Boxer, and DeLeeuw (40) described an infant with a severe and early presentation of cardiomyopathy and hypoketotic hypoglycemia associated with low plasma carnitine, virtually all of which was in the long-chain esterified fraction. They demonstrated a deficiency of the enzyme responsible for shuttling carnitine and acylcarnitine across the inner mitochondrial membrane in fibroblasts. Parents had half-normal levels of TRANS, suggesting autosomal inheritance of this defect. There is no molecular information available about human TRANS deficiency, although some of the properties of this carnitine carrier in rat liver mitochondria have been reported (7).

SCHAD deficiency. A single patient has been described (41) with SCHAD deficiency expressed in muscle, but not in fibroblasts. This child had episodes of myoglobinuria associated with hypoglycemic encephalopathy, during which she excreted medium-chain dicarboxylic acids in urine. Parents of this patient were not studied; hence, it cannot yet be said whether or how this defect is inherited.

2,4-Dienoyl-CoA reductase deficiency. A single patient has been described (38) with a deficiency of this enzyme in postmortem liver and muscle; fibroblasts were unavailable for study. She was hypotonic from birth and died at 4 months of age. There was no abnormal organic aciduria, but plasma carnitine was low and there was increased urinary excretion of 2-*trans*, 4-*cis*-decadienoylcarnitine. This is the first report of an enzyme defect in the β-oxidation of unsaturated fatty acids. Parents were not studied; hence, the inherited nature of this defect remains to be proven.

Riboflavin-responsive β-oxidation defects. There is a group of patients with clinical and laboratory evidence of a defect in fatty acid oxidation, which is highly responsive to riboflavin therapy (85). Lipid storage myopathy associated with an organic aciduria often found in ETF deficiency has been documented in a few cases; reversal of both the myopathy and the abnormal organic acid profile occurs upon treatment with pharmacologic doses of riboflavin. In muscle mitochondria from a 12-year-old girl with such a lipid storage myopathy (98), the activities of MCAD and SCAD were about one-third of normal levels; immunoblot analysis of muscle mitochondria demonstrated the absence of SCAD and reduced amounts of MCAD protein. After oral riboflavin administration, SCAD antigen and activity were restored (98). Similar results were obtained in another patient with riboflavin-responsive myopathy (99). These data are comparable to those previously reported in the riboflavin-deficient rat (100, 101). Patients with this disorder may become riboflavin-deficient due to a defect of riboflavin transport or metabolism; to date, however, such a defect has not been identified. Family studies have not been reported, so it is not yet clear whether this is an inherited disorder.

CONCLUSIONS

A dozen separate defects of mitochondrial β-oxidation have been described, which represent about half of the potential sites for genetic error in this pathway. There are numerous reports of patients in whom a defect of fatty acid oxidation is suspected on clinical and laboratory grounds; this suspicion is often supported by the direct demonstration of defective fatty acid oxidation in fibroblasts (42, 102), but they do not have one of the documented defects. It is likely that these patients have as yet undescribed, inherited disorders of β-oxidation, and it may be simply a matter of time before an accurate diagnosis is made. The very recent description of a new enzyme of mammalian β-oxidation, VLCAD (3), as well as the demonstration that three enzymes of long-chain β-oxidation reside in a single trifunctional protein (15, 95), will surely stimulate the recognition of new disorders in this group of patients.

While considerable new information is available about the molecular aspects of MCAD deficiency, much remains to be learned about the other disorders of β-oxidation at the molecular level. The emergence of this information will lead to improved understanding of the pathogenesis of these disorders, and may provide the tools for the efficient and reliable diagnosis of patients with β-oxidation defects.

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REFERENCES

1. Schulz, H. 1990. Mitochondrial β-oxidation. *In* Fatty Acid Oxidation: Clinical, Biochemical, and Molecular Aspects. K. Tanaka and P. M. Coates, editors. Alan R. Liss, New York, NY. 23–36.
2. Ikeda, Y., K. Okamura-Ikeda, and K. Tanaka. 1985. Purification and characterization of short chain, medium chain and long chain acyl-CoA dehydrogenases from rat liver mitochondria. Isolation of the holo- and apoenzymes and conversion of the apoenzyme to the holoenzyme. *J. Biol. Chem.* **260:** 1311–1325.
3. Izai, K., Y. Uchida, T. Orii, S. Yamamoto, and T. Hashimoto. 1992. Novel fatty acid β-oxidation enzymes in

rat liver mitochondria. I. Purification and properties of very-long-chain acyl-coenzyme A dehydrogenase. *J. Biol. Chem.* **267:** 1027–1033.
4. Kilponen, J. M., P. M. Palosaari, and J. K. Hiltunen. 1990. Occurrence of a long-chain Δ^3, Δ^2-enoyl-CoA isomerase in rat liver. *Biochem. J.* **269:** 223–226.
5. Frerman, F. E. 1988. Acyl-CoA dehydrogenases, electron transfer flavoprotein, and electron transfer flavoprotein dehydrogenase. *Biochem. Soc. Trans.* **16:** 416–418.
6. Zammit, V. A., C. G. Corstorphine, and M. G. Kelliher. 1988. Evidence for distinct functional molecular sizes of carnitine palmitoyltransferases I and II in rat liver mitochondria. *Biochem. J.* **250:** 415–420.
7. Indiveri, C., A. Tonazzi, G. Prezioso, and F. Palmieri. 1991. Kinetic characterization of the reconstituted carnitine carrier from rat liver mitochondria. *Biochim. Biophys. Acta.* **1065:** 231–238.
8. Finocchiaro, G., F. Taroni, M. Rocchi, A. Liras Martin, I. Colombo, G. Torri Tarelli, and S. DiDonato. 1991. cDNA cloning, sequence analysis, and chromosomal localization of the gene for human carnitine palmitoyltransferase. *Proc. Natl. Acad. Sci. USA.* **88:** 661–665.
9. Indo, Y., T. Yang-Feng, R. Glassberg, and K. Tanaka. 1991. Molecular cloning and nucleotide sequence of cDNAs encoding human long chain acyl-CoA dehydrogenase and assignment of the location of its gene (*ACADL*) to chromosome 2. *Genomics.* **11:** 609–620.
10. Matsubara, Y., Y. Indo, E. Naito, H. Ozasa, R. Glassberg, J. Vockley, Y. Ikeda, J. P. Kraus, and K. Tanaka. 1989. Molecular cloning and nucleotide sequence of cDNAs encoding the precursors of rat long chain acyl-coenzyme A, short chain acyl-coenzyme A and isovaleryl-coenzyme A dehydrogenases: sequence homology of four enzymes of the acyl-CoA dehydrogenase family. *J. Biol. Chem.* **264:** 16321–16331.
11. Finocchiaro, G., M. Ito, and K. Tanaka. 1987. Purification and properties of short chain acyl-CoA, medium chain acyl-CoA, and isovaleryl-CoA dehydrogenases from human liver. *J. Biol. Chem.* **262:** 7982–7989.
12. Kelly, D. P., J-J. Kim, J. J. Billadello, B. E. Hainline, T. W. Chu, and A. W. Strauss. 1987. Nucleotide sequence of medium-chain acyl-CoA dehydrogenase mRNA and its expression in enzyme-deficient human tissue. *Proc. Natl. Acad. Sci. USA.* **84:** 4068–4072.
13. Matsubara, Y., J. P. Kraus, H. Ozasa, R. Glassberg, G. Finocchiaro, Y. Ikeda, J. Mole, L. E. Rosenberg, and K. Tanaka. 1987. Molecular cloning and nucleotide sequence of cDNA encoding the entire precursor of rat liver medium chain acyl coenzyme A dehydrogenase. *J. Biol. Chem.* **262:** 10104–10108.
14. Naito, E., H. Ozasa, Y. Ikeda, and K. Tanaka. 1989. Molecular cloning and nucleotide sequence of complementary cDNAs encoding human short chain acyl-CoA dehydrogenase and the study of the molecular basis of short chain acyl-CoA dehydrogenases deficiency. *J. Clin. Invest.* **83:** 1605–1613.
15. Uchida, Y., K. Izai, T. Orii, and T. Hashimoto. 1992. Novel fatty acid β-oxidation enzymes in rat liver mitochondria. II. Purification and properties of enoyl-coenzyme A (CoA) hydratase/3-hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase trifunctional protein. *J. Biol. Chem.* **267:** 1034–1041.
16. Mori, M., Y. Amaya, H. Arakawa, and M. Takiguchi. 1990. Biosynthesis and mitochondrial import of fatty acid oxidation enzymes. *In* Fatty Acid Oxidation: Clinical, Biochemical, and Molecular Aspects. K. Tanaka and P. M. Coates, editors. Alan R. Liss, New York, NY. 663–672.
17. Minami-Ishii, N., S. Takesani, T. Osumi, and T. Hashimoto. 1989. Molecular cloning and sequence analysis of the cDNA for rat mitochondrial enoyl-CoA hydratase. Structural and evolutionary relationships linked to the bifunctional enzyme of the peroxisomal β-oxidation system. *Eur. J. Biochem.* **185:** 73–78.
18. Yamaguchi, S., T. Fukao, H. Nagasawa, T. Orii, N. Sakura, R. B. H. Schutgens, L. Sweetman, Y. Fujiki, K. Kamijo, T. Osumi, and T. Hashimoto. 1990. 3-Ketothiolase deficiency: molecular heterogeneity of the enzyme defect and cloning of the cDNA. *In* Fatty Acid Oxidation: Clinical, Biochemical, and Molecular Aspects. K. Tanaka and P. M. Coates, editors. Alan R. Liss, New York, NY. 673–679.
19. Palosaari, P. M., M. Vihinen, P. I., Mantsala, S. E. H. Alexson, T. Pihlajaniemi, and J. K. Hiltunen. 1991. Amino acid sequence similarities of the mitochondrial short-chain Δ^3, Δ^2-enoyl-CoA isomerase and peroximal Δ^3, Δ^2-enoyl-CoA isomerase, 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase enzyme in rat liver. *J. Biol. Chem.* **266:** 10750–10753.
20. Dommes, V., and W-H. Kunau. 1984. 2,4-Dienoyl coenzyme A reductase from bovine liver and *Escherichia coli*. Comparison of properties. *J. Biol. Chem.* **259:** 1781–1788.
21. Ikeda, Y., and K. Tanaka. 1986. Biosynthesis and mitochondrial processing of electron transfer flavoprotein: a defect in the α-subunit synthesis is a primary lesion in glutaric aciduria type II. *J. Clin. Invest.* **78:** 997–1002.
22. Finocchiaro, G., M. Ito, Y. Ikeda, and K. Tanaka. 1988. Molecular cloning and nucleotide sequence of cDNAs encoding the α-subunit of human electron transfer flavoprotein. *J. Biol. Chem.* **265:** 15773–15780.
23. Finocchiaro, G., N. Archidiacono, C. Gellera, W. Bloisi, I. Colombo, G. Valdameri, G. Romeo, K. Tanaka, and S. DiDonato. 1989. Molecular cloning and chromosomal localization of the β-subunit of human electron transfer flavoprotein (ETF). *Am. J. Hum. Genet.* **45:** A185.
24. Frerman, F. E., and S. I. Goodman. 1985. Deficiency of electron transfer flavoprotein or electron transfer flavoprotein:ubiquinone oxidoreductase in glutaric acidemia type II fibroblasts. *Proc. Natl. Acad. Sci. USA.* **82:** 4517–4520.
25. Goodman, S. I., K. F. Bemelen, and F. E. Frerman. 1992. Human cDNA encoding ETF dehydrogenase (ETF:ubiquinone oxidoreductase), and mutations in glutaric acidemia type II. *In* New Developments in Fatty Acid Oxidation. P. M. Coates and K. Tanaka, editors. Wiley-Liss, New York, NY. In press.
26. Glick, B. S., and G. Schatz. 1991. Import of proteins into mitochondria. *Annu. Rev. Genet.* **25:** 21–44.
27. Ikeda, Y., S. M. Keese, W. A. Fenton, and K. Tanaka. 1987. Biosynthesis of rat liver mitochondrial short chain acyl-CoA, medium chain acyl-CoA, long chain acyl-CoA, and isovaleryl-CoA dehydrogenases: in vitro synthesis, import into mitochondria, and processing of their precursors in a cell-free system and in cultured cells. *Arch. Biochem. Biophys.* **252:** 662–674.
28. DiMauro, S., and P. M. M. DiMauro. 1973. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. *Science.* **182:** 929–931.
29. Bougneres, P-F., J-M. Saudubray, C. Marsac, O. Bernard, M. Odievre, and J. R. Girard. 1980. Decreased ketogenesis due to deficiency of hepatic carnitine acyl transferase. *N. Engl. J. Med.* **302:** 123–124.
30. Kolvraa, S., N. Gregersen, E. Christensen, and N.

Hobolth. 1982. In vitro fibroblast studies in a patient with C_6–C_{10}-dicarboxylic aciduria: evidence for a defect in general acyl-CoA dehydrogenase. *Clin. Chim. Acta.* **126:** 53–67.
31. Rhead, W. J., B. A. Amendt, K. S. Fritchman, and S. J. Felts. 1983. Dicarboxylic aciduria: deficient [1-^{14}C] octanoate oxidation and medium-chain acyl-CoA dehydrogenase in fibrobasts. *Science.* **221:** 73–75.
32. Stanley, C. A., D. E. Hale, P. M. Coates, C. L. Hall, B. E. Corkey, W. Yang, R. I. Kelley, E. L. Gonzales, J. R. Williamson, and L. Baker. 1983. Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels. *Pediatr. Res.* **17:** 877–884.
33. Hale, D. E., M. Batshaw, P. M. Coates, F. E. Frerman, S. I. Goodman, I. Singh, and C. A. Stanley. 1985. Long-chain acyl coenzyme A dehydrogenase deficiency: an inherited cause of non-ketotic hypoglycemia. *Pediatr. Res.* **19:** 666–671.
34. Amendt, B. A., C. Greene, L. Sweetman, J. Cloherty, V. Shih, A. Moon, L. Teel, and W. J. Rhead. 1987. Short-chain acyl-CoA dehydrogenase deficiency: clinical and biochemical studies in two patients. *J. Clin. Invest.* **79:** 1303–1309.
35. Hale, D. E., C. Thorpe, K. Braat, J. H. Wright, C. R. Roe, P. M. Coates, T. Hashimoto, and A. M. Glasgow. 1990. The L-3-hydroxyacyl-CoA dehydrogenase deficiency. *In* Fatty Acid Oxidation: Clinical, Biochemical, and Molecular Aspects. K. Tanaka and P. M. Coates, editors. Alan R. Liss, New York, NY. 503–510.
36. Eriksson, B. O., S. Lindstedt, and I. Nordin. 1988. Hereditary defect in carnitine membrane transport is expressed in skin fibroblasts. *Eur. J. Pediatr.* **147:** 662–663.
37. Treem, W. R., C. A. Stanley, D. N. Finegold, D. E. Hale, and P. M. Coates. 1988. Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts. *N. Engl. J. Med.* **319:** 1331–1336.
38. Roe, C. R., D. S. Millington, D. L. Norwood, N. Kodo, H. Sprecher, B. S. Mohammed, M. Nada, H. Schulz, and R. McVie. 1990. 2,4-Dienoyl-coenzyme A reductase deficiency: a possible new disorder of fatty acid oxidation. *J. Clin. Invest.* **85:** 1703–1707.
39. Demaugre, F., J-P. Bonnefont, M. Colonna, C. Cepanec, J-P. Leroux, and J-M. Saudubray. 1991. Infantile form of carnitine palmitoyltransferase deficiency with hepatomuscular symptoms and sudden death. Physiopathological approach to carnitine palmitoyltransferase II deficiencies. *J. Clin. Invest.* **87:** 859–864.
40. Stanley, C. A., J. Boxer, and S. DeLeeuw. 1991. Mitochondrial inner membrane acylcarnitine translocase deficiency in an infant with an inborn error of fatty acid oxidation. *Pediatr. Res.* **29:** 199A.
41. Tein, I., D. C. DeVivo, D. E. Hale, J. T. R. Clarke, H. Zinman, R. Laxer, A. Shore, and S. DiMauro. 1991. Short-chain L-3-hydroxacyl-CoA dehydrogenase deficiency in muscle: a new cause for recurrent myoglobinuria and encephalopathy. *Ann. Neurol.* **30:** 415–419.
42. Roe, C. R., and P. M. Coates. 1989. Acyl-CoA dehydrogenase deficiencies. *In* The Metabolic Basis of Inherited Disease. 6th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York, NY. 889–914.
43. Matsubara, Y., K. Narisawa, S. Miyabayashi, K. Tada, P. M. Coates, C. Bachmann, L. J. Elsas, R. J. Pollitt, W. J. Rhead, and C. R. Roe. 1990. Identification of a common mutation in patients with medium-chain acyl-CoA dehydrogenase deficiency. *Biochem. Biophys. Res. Commun.* **171:** 498–505.
44. Yokota, I., Y. Indo, P. M. Coates, and K. Tanaka. 1990. The molecular basis of medium-chain acyl-coenzyme A dehydrogenase deficiency: an A to G transition at position 985 that causes a lysine-304 to glutamate substitution in the mature protein is the single prevalent mutation. *J. Clin. Invest.* **86:** 1000–1003.
45. Kelly, D. P., A. J. Whelan, M. L. Ogden, R. Alpers, Z. Zhang, G. Bellus, N. Gregersen, L. Dorland, and A. W. Strauss. 1990. Molecular characterization of inherited medium-chain acyl-CoA dehydrogenase deficiency. *Proc. Natl. Acad. Sci. USA.* **97:** 9236–9240.
46. Gregersen, N., B. S. Andresen, P. Bross, V. Winter, N. Rudiger, S. Engst, E. Christensen, D. Kelly, A. W. Strauss, S. Kolvraa, L. Bolund, and S. Ghisla. 1991. Molecular characterization of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: identification of a lys^{329} to glu mutation in the MCAD gene, and expression of inactive mutant enzyme protein in *E. coli. Hum. Genet.* **86:** 545–551.
47. Curtis, D., A. I. F. Blakemore, P. C. Engel, D. Macgregor, G. Besley, S. Kolvraa, and N. Gregersen. 1991. Heterogeneity for mutations in medium chain acyl-CoA dehydrogenase deficiency in the UK population. *Clin. Genet.* **40:** 283–286.
48. Yokota, I., P. M. Coates, D. E. Hale, P. Rinaldo, and K. Tanaka. 1991. Molecular survey of a prevalent mutation, ^{985}A-to-G transition, and identification of five infrequent mutations in the medium-chain acyl-CoA dehydrogenase (MCAD) gene in 55 patients with MCAD deficiency. *Am. J. Hum. Genet.* **49:** 1280–1291.
49. Blakemore, A. I. F., H. Singleton, R. J. Pollitt, P. C. Engel, S. Kolvraa, N. Gregersen, and D. Curtis. 1991. Frequency of the G985 MCAD mutation in the general population. *Lancet.* **337:** 298–299.
50. Matsubara, Y., K. Narisawa, K. Tada, H. Ikeda, Y. Ye-Qi, D. M. Danks, A. Green, and E. R. B. McCabe. 1991. Prevalence of K329E mutation in medium-chain acyl-CoA dehydrogenase gene determined from Guthrie cards. *Lancet.* **338:** 552–553.
51. Gregersen, N., A. I. F. Blakemore, V. Winter, B. Andresen, S. Kolvraa, L. Bolund, D. Curtis, and P. C. Engel. 1991. Specific diagnosis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in dried blood spots by a polymerase chain reaction (PCR) assay detecting a point mutation (G985) in the MCAD gene. *Clin. Chim. Acta.* **202:** 23–34.
52. Rinaldo, P., J. J. O'Shea, P. M. Coates, D. E. Hale, C. A. Stanley, and K. Tanaka. 1988. Medium chain acyl-CoA dehydrogenase deficiency: diagnosis by stable isotope dilution analysis of urinary n-hexanoylglycine and 3-phenylpropionylglycine. *N. Engl. J. Med.* **319:** 1308–1313.
53. Millington, D. S., D. L. Norwood, N. Kodo, C. R. Roe, and F. Inoue. 1989. Application of fast atom bombardment with tandem mass spectrometry and liquid chromatography/mass spectrometry to the analysis of acylcarnitines in human urine, blood and tissue. *Anal. Biochem.* **180:** 331–339.
54. Duran, M., L. Bruinvis, D. Ketting, J. B. C. de Klerk, and S. K. Wadman. 1988. *Cis*-4-decenoic acid in plasma: a characteristic metabolite in medium-chain acyl-CoA dehydrogenase deficiency. *Clin. Chem.* **34:** 548–551.
55. Coates, P. M., D. E. Hale, C. A. Stanley, B. E. Corkey, and J. A. Cortner. 1985. Genetic deficiency of medium-

chain acyl CoA dehydrogenase: studies in cultured skin fibroblasts and peripheral mononuclear leukocytes. *Pediatr. Res.* **19:** 671–676.
56. Frerman, F. E., and S. I. Goodman. 1985. Fluorometric assay of acyl-CoA dehydrogenases in normal and mutant human fibroblasts. *Biochem. Med.* **33:** 38–44.
57. Matsubara, Y., J. P. Kraus, T. L. Yang-Feng, U. Francke, L. E. Rosenberg, and K. Tanaka. 1986. Molecular cloning of cDNAs encoding rat and human medium-chain acyl-CoA dehydrogenase and assignment of the gene to human chromosome 1. *Proc. Natl. Acad. Sci. USA.* **83:** 6543–6547.
58. Zhang, Z., D. P. Kelly, J-J. Kim, Y. Zhou, M. L. Ogden, A. J. Whelan, and A. W. Strauss. 1992. Structural organization and regulatory regions of the human medium-chain acyl-CoA dehydrogenase gene. *Biochemistry.* **31:** 81–89.
59. Workshop on Molecular Aspects of MCAD Deficiency. 1992. Mutations causing medium-chain acyl-CoA dehydrogenase deficiency: a collaborative compilation of the data from 172 patients. *In* New Developments in Fatty Acid Oxidation. P. M. Coates and K. Tanaka, editors. Wiley-Liss, New York, NY. 449–456.
60. Ikeda, Y., D. E. Hale, S. M. Keese, P. M. Coates, and K. Tanaka. 1986. Biosynthesis of variant medium chain acyl-CoA dehydrogenase in cultured fibroblasts from patients with medium chain acyl-CoA dehydrogenase deficiency. *Pediatr. Res.* **20:** 843–847.
61. Coates, P. M., Y. Indo, D. Young, D. E. Hale, and K. Tanaka. 1992. Immunochemical characterization of variant medium-chain acyl-CoA dehydrogenase in fibroblasts from patients with medium-chain acyl-CoA dehydrogenase deficiency. *Pediatr. Res.* **31:** 34–38.
62. Inagaki, T., N. Ohishi, C. Bachmann, S. Ghisla, N. Tsukagoshi, S. Udaka, and K. Yagi. 1990. Immunochemical and molecular analysis of medium-chain acyl CoA dehydrogenase deficiency. *J. Clin. Biochem. Nutr.* **8:** 1–8.
63. Ogilvie, I., S. Jackson, K. Bartlett, and D. M. Turnbull. 1991. Immunoreactive enzyme protein in medium-chain acyl-CoA dehydrogenase deficiency. *Biochem. Med. Metab. Biol.* **46:** 373–379.
64. Kim, J-J. P., and J. Wu. 1988. Structure of medium-chain acyl-CoA dehydrogenase from pig liver mitochondria at 3-A resolution. *Proc. Natl. Acad. Sci. USA.* **85:** 6677–6681.
65. Kim, J-J. P. 1991. Crystallographic studies of medium-chain acyl-CoA dehydrogenase from pig liver mitochondria. *In* Flavins and Flavoproteins 1990. B. Curti, S. Ronchi, and G. Zanetti, editors. W. deGruyter, Berlin New York. 291–298.
66. Kolvraa, S., N. Gregersen, A. I. F. Blakemore, A. K. Schneidermann, V. Winter, B. S. Andresen, D. Curtis, P. C. Engel, P. Divry, W. Rhead, and L. Bolund. 1991. The most common mutation causing medium-chain acyl-CoA dehydrogenase deficiency is strongly associated with a particular haplotype in the region of the gene. *Hum. Genet.* **87:** 425–428.
67. Kidd, J. R., Y. Matsubara, C. M. Castiglione, K. Tanaka, and K. K. Kidd. 1990. The locus for the medium-chain acyl-CoA dehydrogenase gene on chromosome 1 is highly polymorphic. *Genomics.* **6:** 89–93.
68. Blakemore, A. I. F., P. C. Engel, and D. Curtis. 1990. BamHI and MspI RFLP's in strong linkage disequilibrium at the medium chain acyl-coenzyme A dehydrogenase locus (ACADM chromosome 1). *Nucleic Acids Res.* **18:** 2838.
69. Kelly, D. P., D. E. Hale, S. L. Rutledge, M. L. Ogden, A. J. Whelan, Z. Zhang, and A. W. Strauss. 1992. Molecular basis of inherited medium-chain acyl-CoA dehydrogenase deficiency causing sudden child death. *J. Inherited Metab. Dis.* **15:** 171–180.
70. Ding, J-H., B-Z. Yang, Y. Bao, C. R. Roe, and Y-T. Chen. 1992. Identification of a new mutation in medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. *Am. J. Hum. Genet.* **50:** 229–233.
71. Indo, Y., P. M. Coates, D. E. Hale, and K. Tanaka. 1991. Immunochemical characterization of variant long-chain acyl-CoA dehydrogenase in cultured fibroblasts from nine patients with long chain acyl-CoA dehydrogenase deficiency. *Pediatr. Res.* **30:** 211–215.
72. Coates, P. M., D. E. Hale, G. Finocchiaro, K. Tanaka, and S. M. Winter. 1988. Genetic deficiency of short-chain acyl-coenzyme A dehydrogenase in cultured fibroblasts from a patient with muscle carnitine deficiency and severe skeletal muscle weakness. *J. Clin. Invest.* **81:** 171–175.
73. Turnbull, D. M., K. Bartlett, D. L. Stevens, K. G. M. M. Alberti, G. J. Gibson, M. A. Johnson, A. J. McCulloch, and H. S. A. Sherratt. 1984. Short-chain acyl-CoA dehydrogenase deficiency associated with a lipid-storage myopathy and secondary carnitine deficiency. *N. Engl. J. Med.* **311:** 1232–1236.
74. Wood, P. A., B. A. Amendt, W. J. Rhead, D. S. Millington, F. Inoue, and D. Armstrong. 1989. Short-chain acyl-coenzyme A dehydrogenase deficiency in mice. *Pediatr. Res.* **25:** 38–43.
75. Barton, D. E., T. L. Yang-Feng, G. Finocchiaro, H. Ozasa, K. Tanaka, and U. Francke. 1987. Short chain acyl-CoA dehydrogenase (ACADS) maps to chromosome 12 (q22-qter) and electron transfer flavoprotein (ETFA) to 15 (q23-q25). *Cytogenet. Cell Genet.* **46:** 577.
76. Naito, E., Y. Indo, and K. Tanaka. 1990. Identification of two variant short chain acyl-coenzyme A dehydrogenase alleles, each containing a different point mutation in a patient with short chain acyl-coenzyme A dehydrogenase deficiency. *J. Clin. Invest.* **85:** 1575–1582.
77. Naito, E., Y. Indo, and K. Tanaka. 1989. Short chain acyl-coenzyme A dehydrogenase (SCAD) deficiency: immunochemical demonstration of molecular heterogeneity due to variant SCAD with differing stability. *J. Clin. Invest.* **84:** 1671–1674.
78. Demaugre, F., J-P. Bonnefont, G. Mitchell, N. Nguyen-Hoang, A. Pelet, M. Rimoldi, S. DiDonato, and J-M. Saudubray. 1988. Hepatic and muscular presentations of carnitine palmitoyl-transferase deficiency: two distinct entities. *Pediatr. Res.* **24:** 308–311.
79. Haworth, J. C., P. M. Coates, F. Demaugre, L. A. Dilling, L. E. Seargeant, S. P. Moroz, F. A. Booth, and S. S. Seshia. 1991. Hepatic carnitine palmityltransferase (CPT I) deficiency: three patients in a Hutterite family. *Pediatr. Res.* **29:** 130A.
80. Tein, I., F. Demaugre, J-P. Bonnefont, and J-M. Saudubray. 1989. Normal muscle CPT_1 and CPT_2 activities in hepatic presentation patients with CPT_1 deficiency in fibroblasts. Tissue specific isoforms of CPT_1? *J. Neurol. Sci.* **92:** 229–245.
81. Taroni, F., E. Verderio, S. Fiorucci, G. Finocchiaro, G. Uziel, and S. DiDonato. 1991. Molecular analysis of carnitine palmitoyltransferase deficiency. *Am. J. Hum. Genet.* **49(Suppl):** 420.
82. Demaugre, F., J-P. Bonnefont, C. Cepanec, J. Scholte, J-M. Saudubray, and J-P. Leroux. 1990. Immunoquantitative analysis of human carnitine palmitoyltransferase I and II defects. *Pediatr. Res.* **67:** 497–500.
83. Frerman, F. E., and S. I. Goodman. 1989. Glutaric aciduria type II and defects of the mitochondrial respira-

tory chain. *In* The Metabolic Basis of Inherited Disease. 6th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York, NY. 915–931.
84. Loehr, J. P., S. I. Goodman, and F. E. Frerman. 1990. Glutaric acidemia type II: heterogeneity of clinical and biochemical phenotypes. *Pediatr. Res.* **27:** 311–315.
85. Rhead, W. J. 1991. Inborn errors of fatty acid oxidation in man. *Clin. Biochem.* **24:** 319–329.
86. Gorelick, R. J., and C. Thorpe. 1986. Electron-transferring flavoprotein from pig kidney: flavin analogue studies. *Biochemistry.* **25:** 7092–7098.
87. Indo, Y., R. Glassberg, I. Yokota, and K. Tanaka. 1991. Molecular characterization of variant α-subunit of electron transfer flavoprotein in three patients with glutaric acidemia type II, and identification of glycine substitution for valine-157 in the sequence of the precursor, producing an unstable mature protein in a patient. *Am. J. Hum. Genet.* **49:** 575–580.
88. Colombo, I., S. DiDonato, M. Volta, C. Gellera, B. Garavaglia, L. Montermini, S. Yamaguchi, S. I. Goodman, F. E. Frerman, and G. Finocchiaro. 1992. Molecular heterogeneity of beta-ETF deficiency in glutaric aciduria type II. *In* New Developments in Fatty Acid Oxidation. P. M. Coates and K. Tanaka, editors. Wiley-Liss, New York, NY. 561–566.
89. Yamaguchi, S., T. Orii, Y. Suzuki, K. Maeda, M. Oshima, and T. Hashimoto. 1991. Newly identified forms of electron transfer flavoprotein deficiency in two patients with glutaric aciduria type II. *Pediatr. Res.* **29:** 60–63.
90. Dionisi Vici, C., A. B. Burlina, E. Bertini, C. Bachmann, M. R. M. Mazziotta, F. Zacchello, G. Sabetta, and D. E. Hale. 1991. Progressive neuropathy and recurrent myoglobinuria in a child with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J. Pediatr.* **118:** 744–746.
91. Jackson, S., K. Bartlett, J. Land, E. R. Moxon, R. J. Pollitt, J. V. Leonard, and D. M. Turnbull. 1991. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr. Res.* **29:** 406–411.
92. Przyrembel, H., C. Jakobs, L. Ijlst, J. B. C. de Klerk, and R. J. A. Wanders. 1991. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J. Inherited Metab. Dis.* **14:** 674–680.
93. Tserng, K-Y., S-J. Jin, D. S. Kerr, and C. L. Hoppel. 1991. Urinary 3-hydroxydicarboxylic acids in pathophysiology of metabolic disorders with dicarboxylic aciduria. *Metabolism.* **46:** 676–682.
94. Jackson, S., R. Singh Kler, K. Bartlett, M. Pourfarzam, A. Aynsley-Green, L. A. Bindoff, and D. M. Turnbull. 1992. Combined defect of long-chain 3-hydroxyacyl-CoA dehydrogenase, 2-enoyl-CoA hydratase and 3-oxoacyl-CoA thiolase. *In* New Developments in Fatty Acid Oxidation. P. M. Coates and K. Tanaka, editors. Wiley-Liss, New York, NY. 327–338.
95. Carpenter, K., R. J. Pollitt, and B. Middleton. 1992. Human liver long-chain 3-hydroxyacyl-coenzyme A dehydrogenase is a multifunctional membrane-bound beta-oxidation enzyme of mitochondria. *Biochem. Biophys. Res. Commun.* **183:** 443–448.
96. Tein, I., D. C. De Vivo, F. Bierman, P. Pulver, L. J. de Meirleir, L. Cvitanovic-Sojat, R. A. Pagon, E. Bertini, C. Dionisi Vici, S. Servidei, and S. DiMauro. 1990. Impaired skin fibrobast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy. *Pediatr. Res.* **28:** 247–255.
97. Stanley, C. A., S. DeLeeuw, P. M. Coates, C. Vianey-Liaud, P. Divry, J-P. Bonnefont, J-M. Saudubray, M. Haymond, F. K. Trefz, G. N. Breningstall, R. S. Wappner, D. J. Byrd, C. Sansaricq, I. Tein, W. Grover, D. Valle, and W. R. Treem. 1991. Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake. *Ann. Neurol.* **30:** 709–716.
98. DiDonato, S., C. Gellera, D. Peluchetti, G. Uziel, A. Antonelli, G. Lus, and M. Rimoldi. 1989. Normalization of short chain acylcoenzyme A dehydrogenase after riboflavin treatment in a girl with multiple acyl coenzyme A dehydrogenase-deficient myopathy. *Ann. Neurol.* **25:** 479–484.
99. Turnbull, D. M., I. M. Shepherd, B. Ashworth, K. Bartlett, M. A. Johnson, M. J. Cullen, S. Jackson, and H. S. A. Sherratt. 1988. Lipid storage myopathy associated with low acyl-CoA dehydrogenase activities. *Brain.* **111:** 815–828.
100. Hoppel, C., J. P. DiMarco, and B. Tandler. 1979. Riboflavin and rat hepatic cell structure and function. Mitochondrial oxidative metabolism in deficiency states. *J. Biol. Chem.* **254:** 4164–4170.
101. Sakurai, T., S. Miyazawa, S. Furuta, and T. Hashimoto. 1982. Riboflavin deficiency and β-oxidation systems in rat liver. *Lipids.* **17:** 598–604.
102. Coates, P. M. 1992. New developments in the diagnosis and investigation of fatty acid oxidation disorders. *Eur. J. Pediatr.* In press.