

Mouse Models for Disorders of Mitochondrial Fatty Acid β -Oxidation

A. Michele Schuler and Philip A. Wood

Abstract

Mitochondrial β -oxidation of fatty acids is vital for energy production in periods of fasting and other metabolic stress. Human patients have been identified with inherited disorders of mitochondrial β -oxidation of fatty acids with enzyme deficiencies identified at many of the steps in this pathway. Although these patients exhibit a range of disease processes, Reye-like illness (hypoketotic-hypoglycemia, hyperammonemia and fatty liver) and cardiomyopathy are common findings. There have been several mouse models developed to aid in the study of these disease conditions. The characterized mouse models include inherited deficiencies of very long-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, short-chain acyl-CoA dehydrogenase, mitochondrial trifunctional protein- α , and medium-/short-chain hydroxyacyl-CoA dehydrogenase. Mouse mutants developed, but presently incompletely characterized as models, include carnitine palmitoyltransferase-1a and medium-chain acyl-CoA dehydrogenase deficiencies. In general, the mouse models of disorders of mitochondrial fatty acid β -oxidation have shown clinical signs that include Reye-like syndrome and cardiomyopathy, and many are cold intolerant. It is expected that these mouse models will provide vital contributions in understanding the mechanisms of disease pathogenesis of fatty acid oxidation disorders and the development of appropriate treatments and supportive care.

Key Words: cardiomyopathy; cold intolerance; fasting intolerance; fatty acid β -oxidation; inborn errors; mouse models; organic aciduria; Reye-like illness

Introduction

In 1908, Garrod presented the concept of “inborn errors of metabolism” in his Croonian lectures. Through his observations of patients with alkaptonuria and his later collaborations with Bateson, an English geneticist in the early 1900s, he deduced that the excretion of homogentistic acid must be an inherited defect. He further extrapolated that this

must be a recessive condition. With Garrod’s work, the concept that an error of metabolism could be an inherited defect was first presented to the scientific community. Later, Garrod went on to redefine the concept of genetic diversity by discussing “chemical individuality” (Beaudet et al. 1995). Of course the concept of heritable traits and chemical individuality clearly extends to other species in addition to human beings.

Animal Models

Animal models play an important part in the study of the pathophysiology of human metabolic disease. Although cell culture is a valuable preliminary tool, it is virtually impossible to model whole animal pathophysiology *in vitro*. The ability to study the underlying mechanisms *in vivo* is particularly important in the study of human metabolic disease. Although it is true that the inherited defect may be limited to a single enzyme in a single pathway, the interactions of many metabolic pathways (including the one that is defective) are what dictate the development of the entire disease phenotype. For instance, in many inborn errors of metabolism, both diet and environmental conditions can act as a trigger for the progression of the phenotype from normal to diseased. These conditions cannot be replicated accurately in cell culture.

During the past 15 yr, there has been a tremendous interest in developing mouse models of human inherited diseases. This interest has been stimulated partly by the wealth of genetic resources available to study mice coupled with the relative ease of working with them regarding short generation time, large litters, and relatively low maintenance costs. The discovery and development of mouse models for study of human disease have been either “genotype driven” or “phenotype driven” (McDonald 2000). The first is both the most precise and efficient, although somewhat technically difficult. Targeted disruption of a selected gene of interest can yield a mouse with the precise mutation (e.g., comparable with the human mutant allele), although many targeted mutations have been null mutations. This disruption is accomplished using embryonic stem (ES¹) cell lines

Both authors are in the Department of Genomics and Pathobiology, Schools of Medicine and Dentistry, University of Alabama at Birmingham, where A. Michele Schuler, D.V.M., is a Graduate Fellow, and Philip A. Wood, D.V.M., Ph.D., is Professor and Chairman.

¹Abbreviations used in this article: (gene symbols are italic; all capital letters denote human; initial cap and lower case letters denote mouse); AFLP, acute fatty liver of pregnancy; CPT-1, carnitine palmitoyltransferase-1; CPT-1a, liver carnitine palmitoyltransferase one (*CPT-1a*, *Cpt-1a*);

grown in culture and genetically manipulated by design (Cappechi 1989). The desired mutation is accomplished through the process of homologous recombination using a gene-targeting vector. Chimeric mice are then produced from the targeted ES cell line, and ultimately heterozygous and homozygous mutant mouse lines can be developed from the germ-line chimera. Because gene targeting via ES cell approach has worked only in mice, this also has been another major factor in the large increase in development of mouse models. Many of the mouse models of fatty acid β -oxidation deficiencies have been generated using this technique.

There are two variations of the “phenotype-driven” approach. The first is simply screening naturally occurring “spontaneous” mutants for the desired phenotype, such as diagnostic metabolites in the serum or urine (Wood et al. 1988). The short-chain acyl-CoA dehydrogenase (SCAD¹)-deficient mouse mutant was discovered in this way (Wood et al. 1989). The second variation of the phenotype-driven approach starts with irradiation or chemical mutagenesis (e.g., ethylnitrosurea), of male mice in which mutations are randomly created and the subsequent offspring are screened to identify those with phenotypes similar to the human phenotype (McDonald 2000). Use of this approach is ambitious inasmuch as there will be significant breeding and phenotyping required to identify the rare new mutant with disease traits of interest, especially those characterized by autosomal recessive inheritance. The rate limiting factors of this approach include the generation of hemizygous or homozygous mutant mice together with the required high throughput for phenotyping of large numbers of mice for abnormal characteristics such as elevated concentrations of blood or urinary metabolites (e.g., amino acids, organic acids, or lipoprotein-cholesterol). Many of these diseases also may provide an overt phenotype such as progressive neurological disease or failure to thrive (Craig 2001; Wood et al. 1988).

From a human disease standpoint, inborn errors of metabolism often are revealed as a severe disease. As soon as a particular “unmasking threshold” has been reached, clinical signs can be severe (Dipple and McCabe 2000). These diseases often manifest as infant morbidity and mortality. The severity of the clinical signs ranges from mild (or even no disease phenotype) to severe, and death is common. In

addition, there is often no unequivocal cure, and the patient must instead rely on supportive care.

For these reasons, the development of animal models for inborn errors of metabolism is vital in the understanding of these disease mechanisms as well as the development of the necessary supportive care. In addition, if a cure is to be developed for these devastating inherited disorders, it will likely involve the development and characterization of an appropriate mouse or other animal model. A comprehensive overview of all of the mouse models of inherited metabolic disease is beyond the scope of this review. There is, however, a recent and excellent summary of most of the available mouse models of inherited metabolic diseases (Craig 2001). In the present review, we focus on mouse models that represent mitochondrial disorders of fatty acid β -oxidation.

Mitochondrial β -Oxidation of Fatty Acids

Complete fatty acid oxidation (FAO¹) is vital to the maintenance of metabolic homeostasis in mammals especially during periods of fasting or starvation. Mitochondrial fatty acid β -oxidation occurs through a series of cyclic steps within the mitochondria (Figure 1). Before mitochondrial β -oxidation, the fatty acid must be first activated to the acyl-CoA form and then transported into the mitochondrial matrix. Fatty acids are presented to the cell either as albumin-conjugated fatty acids in the blood or as fatty acids derived from lipolysis of triglycerides in lipoproteins by lipoprotein lipase. Fatty acids are first bound by fatty acid binding proteins and then “activated” to the acyl-CoA form in the cytosol by acyl-CoA synthases that are associated with the endoplasmic reticulum or the outer mitochondrial membrane.

After activation of the fatty acid, the acyl-CoA is transported across the double mitochondrial membrane as an acyl-carnitine via a process requiring carnitine palmitoyltransferase-1 (CPT-1¹), carnitine/acyl-carnitine translocase (CT¹), and carnitine palmitoyltransferase-2 (CPT-2¹) (Roe and Ding 2001). The two isoforms of CPT-1 are known as the liver isoform (CPT-1a¹) and the muscle isoform (CPT-1b¹) (van der Leij et al. 2000).

Carnitine-dependent transport of the long-chain acyl-CoA occurs in four steps. First, carnitine accepts the acyl group of the fatty acid via CPT-1. Second, CT translocates the acyl-carnitine across the outer and inner mitochondrial membranes. Then, CPT-2 catalyzes the carnitine release from the acyl group, which is then reattached to a CoA from within the mitochondrial matrix forming the acyl-CoA that will be β -oxidized. Finally, CT, which mediated the transfer of the acyl-carnitine, simultaneously exchanges free carnitine back into the cytosol.

In a complete cycle of β -oxidation (Figure 1), fatty acids are catabolized to generate an acetyl-CoA and an acyl-CoA, which is two carbon units shorter. The first reaction within the mitochondrial matrix is the acyl-CoA dehydrogenation

CPT-1b, muscle carnitine palmitoyltransferase one (*CPT-1b*, *Cpt-1b*); CT, carnitine/acyl-carnitine translocase; CPT-2, carnitine palmitoyltransferase-2 (*CPT-2*, *Cpt-2*); ES, cell embryonic stem cell; FAO, fatty acid oxidation; HELLP, hemolysis, elevated liver enzymes, low platelets; LCAD, long-chain acyl-CoA dehydrogenase (*ACADL*, *Acadl*); LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase (*ACADM*, *Acadm*); M/SCHAD, medium-/short-chain 3-hydroxyacyl-CoA dehydrogenase; MTP- α , mitochondrial trifunctional protein- α (*HADHA*, *Hadha*); MTP- β , mitochondrial trifunctional protein- β (*HADHB*, *Hadhb*); SCAD, short-chain acyl-CoA dehydrogenase (*ACADS*, *Acads*); VLCAD very long-chain acyl-CoA dehydrogenase (*ACADVL*, *Acadvl*).

Mitochondrial Fatty Acid Oxidation

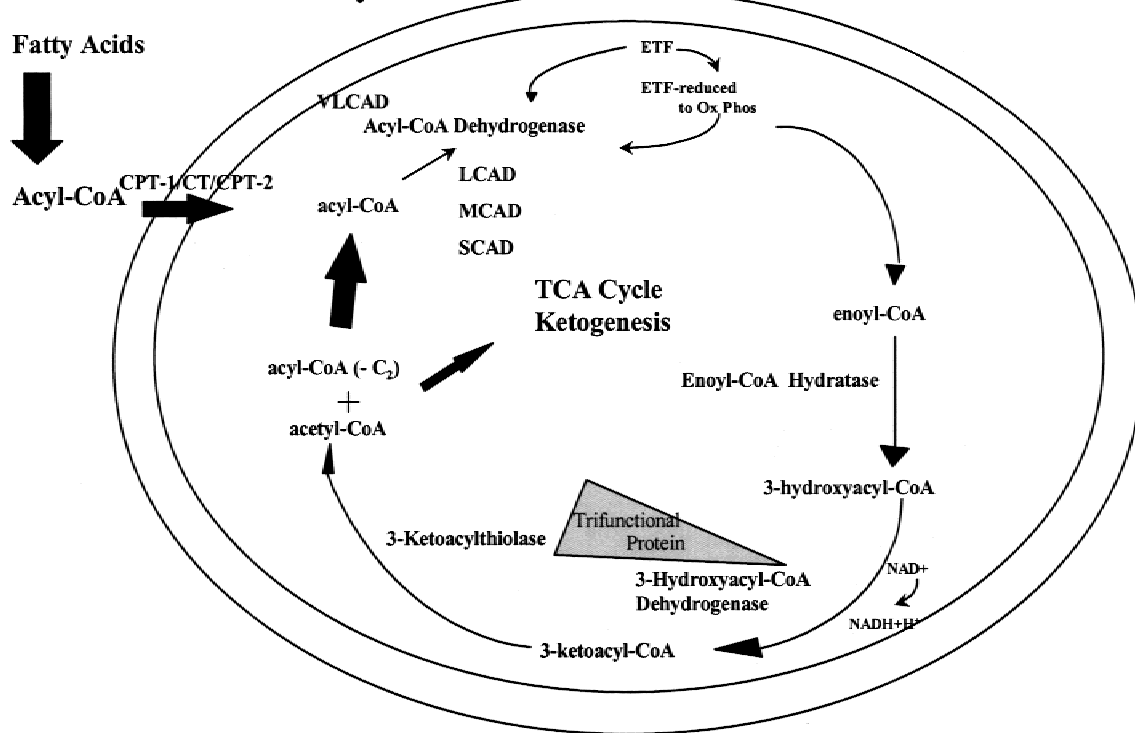


Figure 1 Overview of mitochondrial β -oxidation of fatty acids. CPT-1, carnitine palmitoyltransferase-1; CPT-2, carnitine palmitoyltransferase-2; CT, carnitine/acyl-carnitine translocase; ETF, electron transport flavoprotein; LCAD, long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; TCA, tricarboxylic acid cycle; VLCAD, very long-chain acyl-CoA dehydrogenase.

step that catalyzes the formation of a trans- α , β double bond. The acyl-CoA dehydrogenase step is catalyzed by a group of enzymes named for the chain length of substrate on which they act: very long-chain acyl-CoA dehydrogenase (VLCAD¹), long-chain acyl-CoA dehydrogenase (LCAD¹), medium-chain acyl-CoA dehydrogenase (MCAD¹), and short-chain acyl-CoA dehydrogenase (SCAD¹). Next, enoyl-CoA hydratase catalyzes the hydration of the double bond, which results in the formation of 3-hydroxyacyl-CoA. This process is followed by the 3-hydroxyacyl-CoA dehydrogenases (long-chain hydroxyacyl-CoA dehydrogenase [LCHAD¹] or medium-/short-chain hydroxyacyl-CoA dehydrogenase [M/SCAHD¹]), which catalyze the formation of 3-ketoacyl-CoA through an NAD⁺/NADH-dependent dehydrogenation of 3-hydroxyacyl-CoA. Finally, 3-ketoacyl-CoA thiolase catalyzes the reformation of the shorter acyl-CoA and the newly generated acetyl-CoA through a thiolysis reaction.

These last three steps for long-chain substrates are accomplished by long-chain mitochondrial trifunctional protein (MTP¹), which is composed of MTP α and β proteins. MTP α and MTP β are encoded by two separate but linked genes, *HADHA* and *HADHB*, respectively. MTP α contains the long-chain enoyl-CoA hydratase and LCHAD activities, whereas, MTP β contains the 3-ketoacyl-CoA thiolase activity (Ibdah et al. 2001).

The products of β -oxidation are used to generate the energy necessary for energy homeostasis. FADH₂ and NADH are reoxidized by oxidative phosphorylation within the mitochondrion for ATP synthesis or heat production. Acetyl-CoA, which is generated, is either passed into the tricarboxylic acid cycle for the production of additional ATP or is used for the production of ketones. Ketogenesis is particularly important as an alternative fuel to the brain and heart during periods of starvation.

Human Disorders of Mitochondrial Fatty Acid β -Oxidation

All inborn errors of mitochondrial fatty acid β -oxidation are characterized by fasting-induced disease episodes of hypoketotic-hypoglycemia, metabolic acidosis, hyperammonemia, and fatty liver (Roe and Ding 2001). Generally, this collection of characteristic clinical findings is termed "Reye-like" illness. These characteristics are coupled with an abnormal urinary organic acid and acyl-carnitine profile originating from the buildup of the offending acyl-CoA that is blocked from complete catabolism. These patients have a varying phenotype ranging from no clinical signs to sudden death (Burton 1998; Pollitt 1989; Roe and Ding 2001).

During metabolic stresses (i.e., fasting, thermal stress,

viral illness) that induce hypoglycemia, a “normal” infant should produce ketone bodies as a combined result of mitochondrial FAO and ketogenesis to provide an alternate fuel to help spare glucose as metabolic requirements increase. In addition, β -oxidation of fatty acids is required to energize gluconeogenesis; thus, in an infant with an inborn error of this pathway, the glycogen stores are quickly depleted during metabolic stress. Due to an inadequate ketogenic response and other yet to be defined mechanisms, this infant would be unable to maintain blood glucose (Bennett and Powell 1994).

Human Mitochondrial Carnitine/Acylcarnitine Transport Defects

Human patients with defects in the mitochondrial carnitine/acyl-carnitine transport process have presented clinically much like other patients with inherited defects of mitochondrial fatty acid β -oxidation (i.e., with clinical signs that include Reye-like illness). Although there have been patients described with CPT1a deficiency, there are no known patients with CPT1b deficiency (Roe and Ding 2001). Overall, patients with CPT 1a deficiency have shown a prominent hepatomegaly with fatty change; however, there was no cardiac disease, and plasma carnitine concentrations often were not elevated. CPT-1a-deficient patients are treated with dietary management strategies such as frequent feedings with medium-chain triglycerides and strict avoidance of fasting (Roe and Ding 2001). Patients with CT deficiency have been characterized by either a severe neonatal onset of Reye-like illness and cardiomyopathy or a milder form with hypoglycemia, but no cardiac disease (Roe and Ding 2001). CPT-2-deficient patients have shown cardiomyopathy (newborns and older infants) and muscle weakness (adults) (Roe and Ding 2001).

Human VLCAD Deficiency

There have been many human patients identified with VLCAD deficiency. These patients commonly present during infancy or childhood with cardiomegaly or skeletal myopathy in addition to the common Reye-like illness (Roe and Ding 2001). Instances of sudden death in infancy also have been attributed to VLCAD deficiency (Mathur et al. 1999). Mutations of the VLCAD locus (*ACADVL*) are heterogeneous (Strauss et al. 1995). Although LCAD deficiency was originally described in human patients, it turned out to be a mistaken diagnosis (Roe and Coates 1997) and most of those patients were eventually shown to be VLCAD deficient (Yamaguchi et al. 1993). Thus, there have been no documented cases of human patients with LCAD deficiency.

Human MCAD Deficiency

MCAD deficiency is the most common of the human acyl-CoA dehydrogenase deficiencies. Disease episodes are

characterized by acute metabolic acidosis, hypoketotic hypoglycemia, hyperammonemia, secondary carnitine deficiency, and medium-chain dicarboxylic aciduria (Roe and Ding 2001). In contrast to VLCAD deficiency, cardiac disease is not a common feature in MCAD-deficient patients. The most common mutation is a 985G>A (K329E) mutation in exon 11 (Roe and Ding 2001). This substitution apparently results in the production of an unstable protein. In Caucasians of northern European descent this allele is extremely common. In populations evaluated in North America, England, and Australia, as many as one in 60 individuals may be carriers of this mutant allele (Matsubara et al. 1992).

Human SCAD Deficiency

SCAD deficiency has been identified in humans. SCAD-deficient patients have a variable phenotype ranging from neonatal death to a single life-threatening episode followed by complete recovery (Roe and Ding 2001). During acute metabolic crisis, SCAD-deficient patients experience metabolic acidosis, hypoketotic-hypoglycemia, and short-chain dicarboxylic aciduria characterized by increased excretion of ethylmalonic and methylsuccinic acids (Roe and Ding 2001). In addition, there have been a few distinct polymorphisms or mutations at the SCAD locus (*ACADS*) associated with a wide variety of clinical disease, all sharing ethylmalonic aciduria as the common characteristic (Gregersen et al. 1998).

Human Mitochondrial Trifunctional Protein and Isolated LCHAD Deficiency

There have been reports of human patients who have MTP deficiency, which includes LCHAD deficiency. In contrast, there have been other patients with isolated LCHAD deficiency (Roe and Ding 2001). LCHAD deficiency, like the other diseases of this pathway, often occurs in infants characterized with Reye-like illness. The most common LCHAD mutation is a 1528G>C (E510Q) (Roe and Ding 2001). These patients also may have hypotonia and cardiomyopathy. In addition, there are several LCHAD-deficient patients described with retinopathy, cirrhosis, and peripheral neuropathy, characteristics not found in other disorders of β -oxidation (Roe and Ding 2001).

LCHAD deficiency has been implicated in acute fatty liver of pregnancy (AFLP¹) and the hemolysis, elevated liver enzymes, low platelets (HELLP¹) syndrome. AFLP has most often presented with jaundice, abdominal pain, vomiting and lethargy. Patients with AFLP have had signs consistent with acute liver and renal failure that resolved after delivery of the fetus. Patients with HELLP syndrome also experienced abdominal pain as well as headache and hematuria (Gracia 2001).

Human M/SCHAD Deficiency

Only a few patients have been identified with M/SCHAD deficiency. These patients had variable signs including Reye like illness, cardiomyopathy, and hepatic failure. One patient presented with respiratory distress (O'Brien et al. 1998).

Synergistic Heterozygosity

A common clinical difficulty in this field arises with the patient who has all the typical clinical signs of a defect in fatty acid β -oxidation such as Reye-like illness, cardiac dysfunction, or fasting intolerance but who has no distinguishable diagnostic findings such a clear-cut pattern of abnormal metabolites or an unequivocal enzyme deficiency. One genetic concept recently proposed by Vockley and colleagues (2000) to explain these cases is the possible situation of "synergistic heterozygosity." In these cases, there would be heterozygous deficiencies in two or more different enzymes within a similar pathway such as fatty acid β -oxidation. Specifically, these patients would suffer from haploinsufficiency of two different enzymes resulting in a net functional deficiency in that metabolic pathway, with a disease severity like that of a homozygous deficiency at a single enzymatic step. The advantage of the range of mouse models described below is that we can develop appropriate genetic combinations to evaluate this putative mechanism of synergistic heterozygosity experimentally.

Overall, the human diseases of mitochondrial β -oxidation of fatty acids remain a challenge to diagnose and treat; thus, understanding them mechanistically is a major goal in the development of mouse models. We find the major common characteristics of disease are the following: a shared clinical scenario of a Reye-like illness in all of the enzyme deficiencies of this pathway; cardiac disease in the defi-

ciency of enzymes required for long-chain fatty acid β -oxidation; exacerbation of virtually all of the acute disease problems by fasting. We believe that mouse models are crucial in the understanding of these diseases.

Mouse Models of Disorders of Fatty Acid β -Oxidation

To our knowledge, other than the mouse models described herein, no other animal models have been developed for the inborn errors of mitochondrial β -oxidation of fatty acids. Mouse models have been generated for several of the steps of mitochondrial β -oxidation of fatty acids with phenotypes representative of the human diseases (Table 1). Additionally, due to their inherent metabolic deficiencies, there are practical issues of husbandry, which we describe below.

Mitochondrial Carnitine/Acyl-carnitine Transport Defects

The genes for mouse CPT-1a (*Cpt-1a*¹), CPT-1b (*Cpt-1b*¹), and CPT-2 (*Cpt-2*¹) have been cloned and mapped (Cox et al. 1998). A mouse model is currently under development for CPT-1a deficiency (unpublished results, Cox KB, Nyman LR, Hoppel CL, Wood PA), although its characterization is as yet incomplete. At the time of this writing, no other reported mouse models have been developed to investigate inherited deficiencies of CPT-1b, CT, or CPT-2.

VLCAD Deficiency

Two mouse models of VLCAD deficiency have been produced independently (Cox et al. 2001; Exil et al. 1998, 1999). Unless otherwise indicated, we describe herein our

Table 1 Mouse models of defects in mitochondrial β -oxidation of fatty acids

Disease	Mouse phenotype	References (see text)
Short-chain acyl CoA dehydrogenase deficiency	Nonketotic hypoglycemia, cold intolerance, and fatty liver, kidney	Guerra et al. 1998; Wood et al. 1989
Long-chain acyl-CoA dehydrogenase deficiency	Sudden death, gestational loss, fatty change of liver and heart, cold intolerance Much like human VLCAD ^a deficiency	Guerra et al. 1998; Kurtz et al. 1998
Very long-chain acyl CoA dehydrogenase deficiency	Milder version of mouse LCAD ^a -/- phenotype	Cox et al. 2001
Mitochondrial trifunctional protein deficiency	Neonatal hypoglycemia, fatty change of liver, necrosis and degeneration cardiac and diaphragmatic myocytes, lethal	Ibdah et al. 2001
Medium-/short-chain 3-hydroxyacyl-CoA dehydrogenase	Fasting and cold intolerant with development of fatty liver and kidney	O'Brien et al. 2000

^aLCAD, long-chain acyl-CoA dehydrogenase, VLCAD, very long-chain acyl-CoA dehydrogenase.

experiences with the model reported by Cox and coworkers (2001).

The VLCAD gene (*Acadvl*) was mapped to mouse chromosome 11 (Cox et al. 1998). The synteny of this region has been found to be similar to that of the human VLCAD locus (*ACADVL*) (Cox et al. 1998). Gene targeting of *Acadvl* was accomplished using a replacement strategy that resulted in deleting exons 7 through 19 and producing a null allele (Cox et al. 2001). In the VLCAD-deficient mouse model, the clinical disease includes mild hepatic steatosis, mild fatty change in the heart in response to fasting, and cold intolerance (Cox et al. 2001; Exil et al. 1998, 1999).

Cold intolerance represents a deficiency in nonshivering thermogenesis, a mechanism in which heat is produced by brown adipose tissue. In brown fat (a tissue present in all ages of mice and in neonatal humans), mitochondrial oxidation can be uncoupled from ATP synthesis. When this occurs, the energy generated is released as heat. Within mitochondrial brown fat, proteins (specifically UCP-1 and UCP-2) allow β -oxidation to proceed without the activation of ATP-synthase. As a result, brown fat can produce large amounts of heat. Although there are various approaches to metabolically stress mouse models of inborn errors of metabolism (fasting, exercise), cold challenge (Guerra et al. 1998) has produced by far the most reproducible phenotype in the fatty acid β -oxidation mutants.

VLCAD $^{-/-}$ mice had a predominant elevation in C₁₆-C₁₈-acylcarnitines in bile and serum (Cox et al. 2001). VLCAD $^{-/-}$ mice did not show a pronounced organic aciduria, elevated fatty acid deposition in liver, gestational loss, or sudden death (Cox et al. 2001), in contrast to LCAD-deficient mice.

LCAD Deficiency

The mouse LCAD gene (*Acadl*) has been cloned, mapped, and characterized (Hinsdale et al. 1995; Kurtz et al. 1998b). Although there are no known human LCAD-deficient patients, we have developed an LCAD-deficient mouse model that exhibits the most severe disease phenotype compared with the mouse models for any of the other acyl-CoA dehydrogenase deficiencies. The LCAD $^{-/-}$ mouse model has a null mutation generated by gene targeting with an insertion type-targeting vector. This vector induced a duplication of exons 3 and 4 of *Acadl*, resulting in a perfectly spliced mRNA containing coding sequence for the double set of exons 3 and 4. This event causes an additional 101 amino acids to be inserted, which results in an unstable protein and no enzyme activity (Kurtz et al. 1998a).

The phenotype of the LCAD-deficient mouse is most like human VLCAD deficiency (Kurtz et al. 1998a). Characteristics include unprovoked sudden death, fasting intolerance, cold intolerance, hypoketotic-hypoglycemia, and marked fatty change of liver and heart. These mice also have a conspicuous gestational loss of both LCAD $^{-/-}$ (40% loss) and LCAD $+/-$ (55% loss) pups in utero (Kurtz et al.

1998a). Although the human LCAD $^{-/-}$ patient remains an enigma, gestational loss as found in the mouse model would be one possible explanation. LCAD $^{-/-}$ mice have a distinctive acyl-carnitine profile in bile, serum, and whole blood of markedly elevated C₁₂-C₁₄ acyl-carnitines in contrast to the pattern found in VLCAD $^{-/-}$ mice (Cox et al. 2001). The LCAD-deficient mouse model also was used in studies of nonshivering thermogenesis (Guerra et al. 1998). LCAD $^{-/-}$ mice are very cold intolerant. Uncoupling protein-1 (UCP-1 Cox et al. 2001) expression is reduced in cold-challenged LCAD mutants. These mice also develop fatty change of brown fat when cold challenged (Guerra et al. 1998).

LCAD $^{-/-}$ mice have been crossed with leptin-deficient (*Lep^{ob}/Lep^{ob}*) obese mice (Ingalls et al. 1990). We expected that the combination of the obese phenotype with LCAD deficiency would exacerbate both phenotypes; however, we found no major differences in severity of phenotype in the double mutants compared with the individual phenotypes (unpublished results, Brix AE, Schuler AM, Wood PA).

The variable disease phenotypes in human patients with inherited deficiencies in the β -oxidation pathway are influenced by background genetics. Thus, although many inborn errors of metabolism are inherited as simple recessive mendelian traits, the phenotypes may show an inheritance pattern much like that of a complex trait (Dipple and McCabe 2000). The mutant mouse models are no exception. As an example, we have noticed a major genetic background influence on the number of LCAD $+/-$ mice born during the development of C57BL/6NTacFBR (B6) and 129S6 (129) congenic lines. There was an extreme deficiency of LCAD $+/-$ mice when the mutant allele was crossed onto the B6 background, whereas we found the expected 50% rate of transmission of the mutant allele when backcrossing onto the 129 background (Wood et al. 2001). Intuitively, this finding suggested the presence of some modifier gene(s) within the B6 genome, which reduced the viability of LCAD $+/-$ fetuses compared with 129 background in parallel matings.

In other studies, LCAD-deficient mice have proven to be a valuable mouse model in the study of triglyceride deposition in the heart (Bjorkegren et al. 2001). In a recent study, crosses were performed between LCAD $^{-/-}$ mice and apolipoprotein B (apoB) overexpressing transgenics. The resulting mice had significant reduction of triglyceride storage in the cardiac muscle compared with the LCAD-deficient (without the apoB transgene) control mice when challenged with a fast. This characteristic indicated that apoB production and lipoprotein secretion can occur in the heart and that this mechanism may be important in the clearance of triglyceride from heart, and especially in disease situations such a LCAD deficiency (Bjorkegren et al. 2001).

MCAD Deficiency

The mouse MCAD gene (*Acadm*) has been cloned, mapped, and well characterized (Tolwani et al. 1994, 1996). Cur-

rently, there is a mouse model for MCAD deficiency in development (unpublished results, RJ Tolwani, DA Hamm, PA Wood), and the characterization of this model is under way.

SCAD Deficiency

The gene for mouse SCAD (*Acads*) has been cloned and characterized previously (Kelly and Wood 1996; Kelly et al. 1993). SCAD deficiency was discovered in the BALB/cByJ strain of mice (Armstrong et al. 1993; Wood et al. 1989). This mutant strain was detected by screening urinary organic acids. The idea to screen this strain came from a brief report by Prochazka and Leiter (1986), who described a null genetic marker variant in this mouse strain for the biochemical marker of chromosome 5 known as butyryl-CoA dehydrogenase (*Bcd-1*), later recognized as SCAD and renamed (*Acads*). Mouse SCAD deficiency resulted from a 278-base pair deletion involving exons 9 and 10 of *Acads* resulting in a complete enzyme deficiency (Hinsdale et al. 1993). The mouse model for SCAD deficiency did not have acute disease without periods of metabolic stress; however, it did manifest many of the biochemical and pathological features of the human disease after fasting (Armstrong et al. 1993; Wood et al. 1989). Young SCAD mutants developed a fatty liver coupled with hypoglycemia when fasted overnight (Wood et al. 1989). In our experience, BALB/cByJ mice persistently exhibited an organic aciduria even when unchallenged. This organic aciduria was characterized by elevated concentrations of ethylmalonic acid, methylsuccinic acid, and *N*-butyrylglycine (Wood et al. 1989). The metabolite pattern seen in urine organic acid analysis of these mice was characteristic of a block in oxidation of short chain fatty acids (C₄-C₆). We also found that metabolic gene expression for the pathways of fatty acid β -oxidation, urea cycle and gluconeogenesis all cyclically fluctuate a great deal during early postnatal development in both SCAD^{+/+} and SCAD^{-/-} mice. These fluctuations may coincide with the inability to compensate for SCAD deficiency resulting in an increased vulnerability to a fasting challenge (Hinsdale et al. 1996). There were significantly deficient responses in gene expression and enzyme activities of urea cycle enzymes in the first few days after birth in challenged SCAD^{-/-} pups (Hinsdale et al. 1996). This reduction may be an important influence in the development of hyperammonemia in these diseases.

SCAD^{-/-} mice are cold intolerant. They rapidly became hypothermic when exposed to 4°C temperatures (Guerra et al. 1998). SCAD^{-/-} mice uniformly experienced an inability to thermoregulate and became hypoglycemic when placed in the cold for more than 1 hr (Guerra et al. 1998). This result can be exacerbated by a short fast before cold challenge. In addition, there were significant effects of SCAD deficiency on regulation of UCP-1 mRNA levels in the cold. There was no difference in UCP-1 mRNA levels of SCAD^{-/-} and control mice at room temperature, but when

cold challenged, UCP-1 mRNA levels were higher in control mice than in SCAD^{-/-} mice. In addition, uncoupling protein-2 (UCP-2) mRNA levels were elevated in both groups of mice when cold challenged, but these levels were higher in SCAD^{-/-} mice than in control mice (Guerra et al. 1998). Therefore, in SCAD^{-/-} mice UCP-2 appeared to be more responsive than UCP-1 (Guerra et al. 1998). Regardless of the mechanisms of cold intolerance of SCAD^{-/-} mice, cold challenge was a sensitive and reproducible method that forced the mouse to use an energy source other than glycogen stores and to rely on FAO (Guerra et al. 1998).

After the successful cloning and characterization of the mouse SCAD gene (*Acads*) (Kelly and Wood 1996), the SCAD-deficient mouse model was used in studies pursuing gene therapy correction of β -oxidation defects. In these studies, we made transgenic lines that overexpressed normal SCAD activity in the liver and were generated on SCAD^{-/-} background (Kelly et al. 1997). Both Northern blot analysis of liver RNA and enzyme analysis for SCAD activity revealed a normal to increased expression of SCAD in these transgenic lines. Phenotypically, these mice had a decreased incidence and severity of fatty liver and a decreased organic aciduria metabolite pattern even after a fast (Kelly et al. 1997). These studies indicated that although SCAD is expressed in many tissues throughout the body, genetic correction in liver alone had a significant therapeutic benefit.

SCAD mutant mice have been used in a variety of other studies including breeding experiments, nutrition studies, and blood lipid analysis (Wood et al. 1999). Qureshi and others (1993) combined the SCAD^{-/-} mouse model with the X-linked sparse-fur mutation (ornithine transcarbamylase deficiency) to investigate the possible synergy of disorders in fatty acid β -oxidation and disorders of the urea cycle. This double mutant had an increased mortality after weaning (Qureshi et al. 1993). Jimenez-Sanchez and colleagues (2000) combined the SCAD^{-/-} mouse with the peroxisomal membrane protein 70 (PMP70)-deficient mouse model. These double mutants had an additive pattern of abnormal urinary organic acid metabolites (Jimenez-Sanchez et al. 2000). In another study, SCAD-deficient mice fed a riboflavin-deficient diet exhibited a more pronounced secondary carnitine deficiency and a propensity toward secondary hyperammonemia (Rao and Qureshi 1997). Park and colleagues (1997) proposed the use of several mouse strains as hyperlipidemic models, of which BALB/cByJ (SCAD^{-/-} mice) were evaluated. Evaluation of blood lipids in SCAD^{-/-} mice revealed elevated serum cholesterol (total and HDL) and triglycerides. This study also found these mice to be significantly heavier (Park et al. 1997).

Mitochondrial Trifunctional Protein and LCHAD Deficiency

Mitochondrial trifunctional protein (MTP¹) constitutes three enzyme activities of fatty acid β -oxidation as described

earlier. A knockout mouse model has been developed for mitochondrial trifunctional protein deficiency (Ibdah et al. 2001) by gene knockout of MTP α gene (*Hadha*). This condition in the mouse is lethal during the neonatal period, and the pathogenesis includes hepatic steatosis, myocyte necrosis, and hypoglycemia. This model also was used to investigate intrauterine growth retardation in conjunction with FAO disorders. MTP-deficient mice have significantly lower birth weights without an increase in intrauterine lethality.

M/SCHAD Deficiency

M/SCHAD knockouts were generated using gene-targeting techniques. Like many other mouse models of mitochondrial fatty acid β -oxidation, these mice are cold and fasting intolerant. They also develop hepatic steatosis (O'Brien et al. 2000). A full description of this mutant has not been published.

Practical Considerations

When maintaining colonies of mice with disorders of FAO, care must be taken to ensure reduced metabolic stress, including variations in ambient temperature (particularly the avoidance of drops in temperature) and periods of inadequate amounts of food. In addition, when considering the shipment of these mice, it is important to take into consideration the time of year (avoid cold temperatures) and the availability of food and water sources. These mouse models are extremely susceptible to any perturbation in homeostasis.

Summary

Overall, there are a number of mouse models for disorders of mitochondrial fatty acid β -oxidation (Wood 1999). Further development and characterization of mouse models of FAO in particular, may help elucidate probable mechanisms and help define improved treatment for intrauterine growth retardation, AFLP, HELLP, sudden death in infants, and Reye-like illness in toddlers.

Acknowledgments

We thank our many colleagues who have contributed to the studies referred to here. Mouse model studies reported from our laboratory were supported by National Institutes of Health grant RO1-RR02599.

References

Armstrong DL, Masiowski ML, Wood PA. 1993. Pathologic characterization of short-chain acyl-CoA dehydrogenase deficiency in BALB/cByJ mice. *Am J Med Genet* 47:884-892.

- Beaudet AL, Scriver CR, Sly WS, Valle D. 1995. Genetics, biochemistry, and molecular basis of variant human phenotypes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 7th ed. New York: McGraw-Hill. p 54.
- Bennett MJ, Powell S. 1994. Metabolic disease and sudden unexpected death in infancy. *Hum Pathol* 25:742-746.
- Björkegren J, Véniant M, Kim SK, Withycombe SK, Wood PA, Hellerstein MK, Neese RA, Young SG. 2001. Lipoprotein secretion and triglyceride stores in the heart. *J Biol Chem* 276:38511-38517.
- Burton BK. 1998. Inborn errors of metabolism in infancy: A guide to diagnosis. *Pediatrics* 102:E69.
- Capecchi MR. 1989. Altering the genome by homologous recombination. *Science* 244:1288-1292.
- Cox KB, Hamm DA, Millington DS, Matern D, Vockley J, Rinaldo P, Pinkert CA, Rhead WJ, Lindsey JR, Wood PA. 2001. Gestational, pathologic, and biochemical differences between very long-chain acyl-CoA dehydrogenase deficiency and long-chain acyl-CoA dehydrogenase deficiency in the mouse. *Hum Mol Genet* 10:2069-2077.
- Cox KB, Johnson KR, Wood PA. 1998. Chromosomal locations of the mouse fatty acid oxidation genes *Cpt1a*, *Cpt1b*, *Cpt2*, *Acadvl*, and metabolically related *Crat* gene. *Mamm Genome* 9:608-610.
- Craigie WJ. 2001. Mouse models of human genetic disorders. In: Scriver CR, Beaudet AL, Valle D, Sly WS, Childs B, Kinzler KW, Vogelstein B, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill. p 379-416.
- Dipple KM, McCabe ER. 2000. Phenotypes of patients with "simple" mendelian disorders are complex traits: Thresholds, modifiers, and systems dynamics. *Am J Hum Genet* 66:1729-1735.
- Exil VJ, Sims H, Kovacs A, Qin W, Boero J, Khuchua Z, Strauss AW. 1998. Physiologic stressors inducing sudden death in the very-long-chain acyl-CoA dehydrogenase deficient mice. *Circulation* 98:1-5.
- Exil VJ, Sims H, Qin W, Boero J, Khuchua Z, Strauss AW. 1999. Stress-induced death in the very-long-chain acyl-CoA dehydrogenase deficient mouse. *Circulation* 100:1-492.
- Gracia PV. 2001. Acute fatty liver and HELLP syndrome: Two distinct pregnancy disorders. *Int J Gynecol Obstet* 73:215-220.
- Gregersen N, Winter VS, Corydon MJ, Corydon TJ, Rinaldo P, Ribes A, Martinez G, Menett MJ, Vianey-Saban C, Bhala A, Hale DE, Lehnert W, Kmoch S, Roig M, Riudor E, Eiberg H, Andersen BS, Bross P, Bolund LA, Kolvraa S. 1998. Identification of four new mutations in the short-chain acyl-CoA dehydrogenase (SCAD) gene in two patients: One of the variant alleles, 511CT, is present at an unexpectedly high frequency in the general population, as was the case for 625GA, together conferring susceptibility to ethylmalonic aciduria. *Hum Mol Genet* 7:619-627.
- Guerra C, Koza RA, Walsh K, Kurtz DM, Wood PA, Kozak LP. 1998. Abnormal nonshivering thermogenesis in mice with inherited defects of fatty acid oxidation. *J Clin Invest* 102:1724-1731.
- Hinsdale ME, Kelly CL, Wood PA. 1993. Null allele at *bcd-1* locus in BALB/cByJ mice is due to a deletion in the short-chain acyl-CoA dehydrogenase gene and results in missplicing of mRNA. *Genomics* 16:605-611.
- Hinsdale ME, Farmer SC, Johnson KR, Davisson MT, Hamm DA, Tolwani RJ, Wood PA. 1995. RNA expression and chromosomal location of the mouse long-chain acyl-CoA dehydrogenase gene. *Genomics* 28:163-170.
- Hinsdale ME, Hamm DA, Wood PA. 1996. Effects of short-chain acyl-CoA dehydrogenase deficiency on developmental expression of metabolic enzyme genes in the mouse. *Biochem Mol Med* 57:106-115.
- Ibdah JA, Paul H, Zhao Y, Binford S, Salleng K, Cline M, Matern D, Bennett MJ, Rinaldo P, Strauss AW. 2001. Lack of mitochondrial trifunctional protein in mice causes neonatal hypoglycemia and sudden death. *J Clin Invest* 107:1403-1409.
- Ingalls AM, Dickie MM, Snell GD. 1950. Obese, a new mutation in the house mouse. *J Hered* 41:317-318.
- Jimenez-Sanchez G, Hebron KJ, Silva-Zolezzi I, Thomas G, Wood PA, Valle D. 2000. Defective nonshivering thermogenesis and dicarboxylic

- aciduria in mice deficient in the 70kDa peroxisomal membrane protein (PMP70). *Pediatr Res* 47:240A.
- Kelly CL, Hinsdale ME, Wood, PA. 1993. Cloning and characterization of mouse short-chain acyl-CoA dehydrogenase cDNA. *Genomics* 18:137-140.
- Kelly CL, Wood PA. 1996. Cloning and characterization of the mouse short-chain acyl-CoA dehydrogenase gene. *Mamm Genome* 7:262-264.
- Kelly CL, Rhead WJ, Kutschke WK, Brix AE, Hamm DA, Pinkert CA, Lindsey JR, Wood PA. 1997. Functional correction of short-chain acyl-CoA dehydrogenase deficiency in transgenic mice: Implications for gene therapy of human mitochondrial enzyme deficiencies. *Hum Mol Genet* 6:1451-1455.
- Kurtz DM, Rinaldo P, Rhead WJ, Tain L, Millington DS, Vockley J, Hamm DA, Brix AE, Lindsey JR, Pinkert CA, O'Brien WE, Wood PA. 1998a. Targeted disruption of a mouse long-chain acyl-CoA dehydrogenase gene reveals crucial roles for fatty acid oxidation. *Proc Natl Acad Sci U S A* 95:15592-15597.
- Kurtz DM, Tolwani, RJ, Wood PA. 1998b. Structural characterization of the mouse long-chain acyl-CoA dehydrogenase gene and 5' regulatory region. *Mamm Genome* 9:361-365.
- Mathur A, Sims HF, Gopalakrishnan D, Gibson B, Rinaldo P, Vockley J, Hug G, Strauss AW. 1999. Molecular heterogeneity in very-long-chain acyl-CoA dehydrogenase deficiency causing pediatric cardiomyopathy and sudden death. *Circulation* 99:1337-1343.
- Matsubara Y, Narisawa K, Tada K, Ikeda H, Yeji Y, Danks DM, Green A, McCabe ER. 1992. Prevalence of K329E mutation in the medium-chain acyl-CoA dehydrogenase gene determined from Guthrie cards. *Prog Clin Biol Res* 375:453-462.
- McDonald JD. 2000. Production of mouse models for the study of human inborn errors of metabolism. *Mol Genet Metab* 71:240-244.
- O'Brien LK, Gibson B, Charrow J, Rinaldo P, Bennett MJ, Strauss AW. 1998. Mutations in the short chain L, 3-hydroxyacyl-CoA dehydrogenase gene. 4th Fatty Acid and Ketogenesis Conference, London, England. p 61.
- O'Brien LK, Sims HF, Bennett MJ, Strauss AW. 2000. A mouse model for medium and short chain L-3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inher Metab Dis* 23(S1):127.
- Park EI, Paisley EA, Mangian HJ, Swartz DA, Wu M, O'Morchoe PJ, Behr SR, Visek WJ, Kaput J. 1997. Lipid level and type alter stearyl CoA desaturase mRNA abundance differently in mice with distinct susceptibilities to diet-influenced diseases. *J Nutr* 127:566-573.
- Prochazka M, Leiter EH. 1986. A null activity variant found at the butyryl CoA dehydrogenase (*Bcd-1*) locus in BALB/cByJ mice. *Mouse News Lett* 78:31.
- Pollitt RJ. 1989. Disorders of mitochondrial β -oxidation: Prenatal and early postnatal diagnosis and their relevance to Reye's syndrome and sudden infant death. *J Inher Metab Dis* 12(S1):215-230.
- Qureshi IA, LeBlanc D, Cyr D, Giguere R, Mitchell G. 1993. Breeding experiments to combine the X-linked sparse-fur (*spf*) mutation with the autosomal recessive BALB/cByJ strain: Testing the biochemical phenotype of double-mutant mice as a model for ammonia: Fatty acyl CoA synergism. *Biochem Biophys Res Commun* 191:744-749.
- Rao KVR, Qureshi IA. 1997. Decompensation of hepatic and cerebral acyl-CoA metabolism in BALB/cByJ mice by chronic riboflavin deficiency: Restoration by acetyl-L-carnitine. *Can J Physiol Pharmacol* 75:423-4360.
- Roe CR, Coates PM. 1997. Mitochondrial fatty acid oxidation disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 7th ed. New York: McGraw-Hill. p 1501-1533. CD-ROM ed.
- Roe CR, Ding J. 2001. Mitochondrial fatty acid oxidation disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler KW, Vogelstein B, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill. p 2297-2326.
- Strauss AW, Powell CK, Hale DE, Anderson MM, Ahuja A, Brackett JC, Sims HF. 1995. Molecular basis of human mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency causing cardiomyopathy and sudden death in childhood. *Proc Natl Acad Sci U S A* 92:10496-10500.
- Tolwani RJ, Farmer SC, Kurtz DM, Johnson KR, Davisson MT, Hinsdale ME, Cresci S, Kelly DP, Wood PA. 1996. Structure and chromosomal location of the mouse medium-chain acyl-CoA dehydrogenase gene and its promoter. *Gene* 170:165-171.
- Tolwani RJ, Farmer SC, Wood PA. 1994. Molecular cloning and characterization of the mouse-medium-chain acyl-CoA dehydrogenase cDNA. *Genomics* 23:247-249.
- van der Leij FR, Huijckman NC, Boomsma C, Kuipers JR, Bartelds B. 2000. Genomics of the human carnitine acyltransferase genes. *Mol Genet Metab* 71:139-153.
- Vockley J, Rinaldo P, Bennett MJ, Matern D, Vladutiu GD. 2000. Synergistic heterozygosity: Disease resulting from multiple partial defects in one or more metabolic pathways. *Mol Genet Metab* 71:10-18.
- Wood PA, Armstrong D, Sauls D, Davisson MT. 1988. Screening mutant mice for inborn errors of metabolism. *Lab Anim Sci* 38:15-19.
- Wood PA, Amendt BA, Rhead WJ, Millington DS, Inoue F, Armstrong D. 1989. Short-chain acyl-coenzyme A dehydrogenase deficiency in mice. *Pediatr Res* 25:38-43.
- Wood, PA. 1999. Defects in mitochondrial β -oxidation of fatty acids. *Curr Opin Lipidol* 10:107-112.
- Wood PA, Kelly-Kurtz L, Hinsdale ME, Hamm DA, Rhead WJ. 1999. Lessons learned from the mouse model of short-chain acyl-CoA dehydrogenase deficiency. In: Quant PA, Eaton S, eds. *Current Views of Fatty Acid Oxidation and Ketogenesis*. New York: Kluwer Academic/Plenum Press. p 395-402.
- Wood PA, Berger PS, Hamm DA, Pinkert CA. 2001. Gestational loss is a complex trait in the long-chain acyl-CoA dehydrogenase deficient mouse model. *Eur J Hum Genet* 9(S1):335.
- Yamaguchi S, Indo Y, Coates PM, Hashimoto T, Tanaka K. 1993. Identification of very-long-chain-acyl-CoA dehydrogenase deficiency in three patients previously diagnosed with long-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 34:111-113.