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# Disorders of Fatty Acid Oxidation in the Era of Tandem Mass Spectrometry in Newborn Screening

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With recent advances in laboratory technology with tandem mass spectrometry (MS/MS), the number of infants identified with a fatty acid oxidation disorder has increased dramatically. Disorders of fatty acid oxidation comprise one of the most rapidly growing groups within the field of errors of metabolism. This review will explore the recent developments in newborn screening related to the use of tandem mass spectrometry and disorders of fatty acid oxidation.

**Keywords:** Fatty acid oxidation; Tandem mass spectrometry

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In 1961, Guthrie and Susi<sup>1</sup> developed a sensitive but simple and inexpensive screening test for phenylketonuria (PKU). This screening test used a bacterial inhibition assay of dried blood collected on filter paper to measure phenylalanine. With early identification of newborns with PKU, early initiation of a diet low in phenylalanine resulted in normal cognitive development rather than severe mental retardation associated with classic untreated PKU.<sup>2</sup> By 1961, Oregon and Massachusetts began screening every newborn in those states for PKU.<sup>3</sup> By the late 1960s, the practice of routine newborn screening for PKU had expanded to almost every state and, soon afterward, to most developed countries. Within three decades, all states and

territories in the United States had instituted universal newborn screening for PKU.<sup>4-7</sup> Subsequently, newborn screening programs have added screening tests for other disorders, including amino acidopathies, galactosemia, congenital hypothyroidism, and sickle cell disease. The disorders were added based upon the principles of newborn screening.<sup>8-10</sup>

Almost all of the disorders screened and identified by newborn screening programs are mendelian or inherited conditions. However, other conditions, such as HIV and congenital toxoplasmosis, have been included in some screening programs.<sup>11,12</sup> For a few of the disorders, the evidence for efficacy of early medical intervention that early screening allowed was overwhelming, as with PKU and congenital hypothyroidism.<sup>2,13</sup> In other cases, the benefits of medical intervention were less dramatic but significant enough to warrant screening.<sup>14,15</sup>

As more disorders were added to the original screening for PKU, the laboratory needs increased because each disorder had a separate assay: universal newborn screening continued to rely on the “one test-one disorder” system. Screening needed to be more efficient and comprehensive. The development of a single assay that could be used to detect several disorders from a single sample was desired. With advances in technology, tandem mass spectrometry (MS/MS) provided a mechanism to screen for multiple metabolic disorders using a single sample.<sup>16,17</sup> This technology can detect an array of metabolic disorders, including amino acids disorders, fatty acid oxidation disorders (FAODs), and organic acidemias during the neonatal period many times before a catastrophic event (Table 1).<sup>18-25</sup> The purpose of this review is to explore the recent developments in the field of newborn screening related to the use of tandem mass spectrometry and disorders of fatty acid oxidation.

# Tandem Mass Spectrometer

## History of Tandem Mass Spectrometer

A tandem mass spectrometer (MS/MS) is one type of analytical instrument used in laboratories. (Fig 1). This instrument can analyze numerous compounds, such as those in body fluids, foods, and pharmaceuticals. Simplistically, the single mass

spectrometer (MS) weighs molecules (Fig 2). As molecules are extremely small and cannot be weighed in the usual method on a scale, the MS weighs molecules electronically and can determine each molecule's unique mass and how much of the compound is present in the material that is being analyzed. A good analogy for this is pocket change. When you grab a handful of coins, you may have pennies, dimes, nickels, and quarters. If you sort these from lightest to heaviest, the dimes would be the lightest, whereas the quarters would be the heaviest. The other coins would fall

**Table 1. Summary of Inborn Errors of Metabolism Detectable Through Tandem Mass Spectrometry During the Neonatal Period**

Amino Acid Disorders	Fatty Acid Disorders	Organic Acid Disorders
Argininemia	Carnitine acylcarnitine translocase deficiency (CACT)	2-Methylbutyryl-coenzyme A dehydrogenase deficiency (2MBG or 2MBCD or SBCAD)
Argininosuccinic acid lyase deficiency (ASA)		2-Methyl-3-OH butyric aciduria (2M3HBA)
Citrullinemia (CIT)	Carnitine palmitoyl transferase deficiency, type I (CPT I)	3-Hydroxy-3-methylglutaryl deficiency (HMG)
Homocystinuria (HCY)		3-Methylcrotonyl-coenzyme A carboxylase deficiency (3MCC)
Hypermethioninemia (MET)	Carnitine palmitoyl transferase deficiency, type II (CPT II)	3-Methylgluta-cronyl-coenzyme A hydratase deficiency (3MGA)
Hyperphenylalaninemas: Classical PKU (PKU), Benign Hyperphenylalaninemia (H-PHE), Biopterin Cofactor Defects (BIOPT)	Carnitine uptake defect/transport deficiency (CUD)	Glutaric acidemia, type I (GA-I) Isobutyrylglycinuria deficiency (IBG) Isovaleric acidemia (IVA)
Maple syrup urine disease (MSUD)	2,4-Dienoyl-coenzyme A reductase deficiency (DE RED)	Malonic acidemia (MAL)
	Long chain acyl-coenzyme A dehydrogenase deficiency (LCAD)	Methylmalonic acidemias (MMA)
	Long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (LCHAD)/ Trifunctional protein deficiency (TFP)	Mitochondrial acetoacetyl-coenzyme A thiolase deficiency (SKAT, BKT, 3-ketothiolase deficiency, $\beta$ ketothiolase)
	Medium chain acyl-coenzyme A dehydrogenase deficiency (MCAD)	Multiple carboxylase deficiency (MCD)
	Medium/Short chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (MCHAD/SCHAD)	Propionic acidemia (PA)
	Medium chain 3-ketoacyl-coenzyme A thiolase deficiency (MCAT)	
	Multiple acyl-coenzyme A dehydrogenase deficiency (MADD or GAIL, Glutaric acidemia, type II)	
	Short chain acyl-coenzyme A dehydrogenase deficiency (SCAD)	
	Very long chain acyl-coenzyme A dehydrogenase deficiency (VLCAD)	

Data from Sweetman L, Millington DS, Therrell BL et al: Naming and counting disorders (conditions) include in newborn screening. *Pediatrics* 2006;117:S308-S314; Millington D, Koeberl D. Metabolic Screening in the newborn. *Growth, Genetics & Hormones* 2003;19(3):33-38; Zytovicz TH, Fitzgerald EF, Marsden D, et al. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a 2-year summary from the New England Newborn Screening Program. *Clinical Chemistry* 2001;47(11):1945-1955; Rinaldo P, Matern D. Disorders of fatty acid transport and mitochondrial oxidation: Challenges and dilemmas of metabolic evaluation. *Genetics in Medicine* 2000;2(6):338-34. Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Seminars in Perinatology* 1999;23(2), 183-193.



**Fig 1.** Tandem mass spectrometer. Reprinted with permission from Waters Corporation (MS Technologies Centre, Milford, MA).

between the dimes and the quarters. The MS sorts molecules by weight in much the same way.

An MS/MS is simply two single MS with a special “collision cell” chamber between the two MS instruments (Fig 3).<sup>26</sup> After being prepped, the sample is injected into the first instrument. While in the first instrument, the sample is ionized to produce molecular ions, which are determined based upon mass-to-charge ( $m/z$ ) ratio. The ionized molecules are sorted and weighed. Next, the sample is sent into the collision cell chamber where the molecular ion sample is broken into fragmented pieces, called analytes, which are like pieces of a puzzle. Then the sample is passed into the second instrument where quantities of the selected analyte(s) are sorted and weighed according to their  $m/z$  ratio. The peak of each analyte is compared with internal standard to yield both a qualitative and quantitative result. The result can be analyzed within minutes to produce histograms for analysis. Returning to the pocket change analogy, pennies are broken down into copper and zinc, whereas nickels, dimes, and quarters into copper and nickel. Analytes of copper, nickel, and zinc would be qualitatively (what kind) and quantitatively (how much) reported, rather

than the groups of dimes, nickels, pennies, and quarters. The MS/MS analysis produces a report on the type and amount of each analyte present in the sample.

## Tandem Mass Spectrometer and Universal Screening

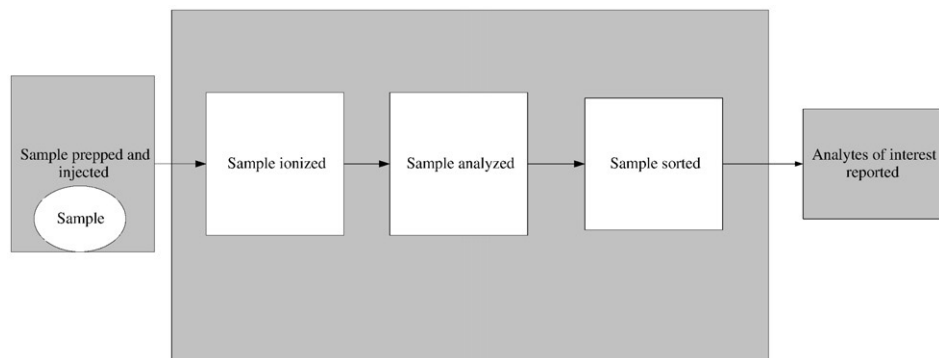
Fatty acid oxidation disorders occur when an enzyme is missing or significantly decreased in the metabolic pathway. A summary of fatty acid disorders detected through MS/MS during the neonatal period is provided in Table 1.<sup>18-20</sup> They are listed according to the length of the fatty acid chain as detected by newborn screening using MS/MS. In a newborn's dried filter paper blood spot, one of the components that MS/MS tests for is acylcarnitines, fats that have been attached to a carnitine.<sup>27</sup> Carnitine is an ammonium compound that has a key role in the transport of fats in and out of the mitochondria, the cell's energy factory.<sup>28</sup> An acylcarnitine is identified by the size of the fat molecule attached to it. These are categorized as short, medium, and long-chain fatty acids and are abbreviated as a combination of letters and numbers (Table 2).

### Diagnosis of Fatty Acid Oxidation by MS/MS

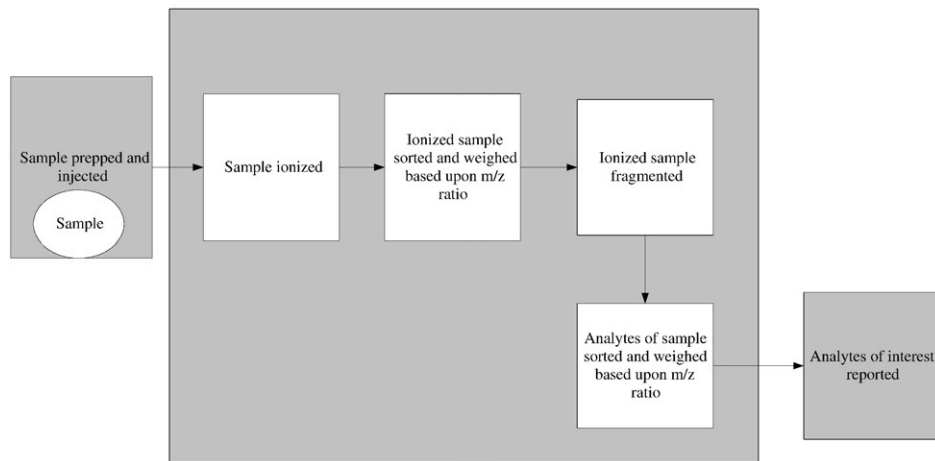
As early as 1984, the diagnostic value of analysis of acylcarnitines was demonstrated and eventually was used to identify medium chain acyl-CoA dehydrogenase (MCAD) deficiency using newborn dried blood spots when C8 (octanoylcarnitine) concentration and a ratio of C8/C10 are greater than four to one.<sup>24,27,29,30</sup> In 2005, a consensus statement was issued to screen without further delay at least for MCAD deficiency in all 50 states.<sup>31</sup>

## Metabolic Effects of Impaired Fatty Acid Metabolism

In FAODs, specific enzymes that are needed in the conversion of fat to energy are either significantly decreased or absent within the normal mitochondrial pathway (Fig 4).<sup>32,33</sup>



**Fig 2.** Simplified schematic of the components of a mass spectrometer. Reprinted from Banta-Wright SA, Steiner RD. Tandem mass spectrometry in newborn screening: A primer for neonatal and perinatal nurses. *J Perinat Neonatal Nurs* 2004;18(1):59-60 with permission from Lippincott Williams & Wilkins.



**Fig 3.** Simplified components of a tandem mass spectrometer. Reprinted from Banta-Wright SA, Steiner RD. Tandem mass spectrometry in newborn screening: A primer for neonatal and perinatal nurses. *J Perinat Neonatal Nurs* 2004;18(1):59-60 with permission from Lippincott Williams & Wilkins.

Usually, these pathways are mediated by coenzyme A (CoA), leading to the formation of acetyl-CoA. When there is a block within the pathway, an abnormal amount of a specific acetyl-CoA begins to accumulate in the blood and tissues of the body, leading to both intoxication and energy deficiency (Fig 5). With the accumulation of fatty acyl-CoA species, the secondary metabolite produces intoxication, such as the accumulation of ammonia. The shortage of acetyl-CoA impairs hepatic ketogenesis and the synthesis of ATP by the oxidation phosphorylation pathway. This results in energy depletion, such as the absence of ketones to extrahepatic tissues.

## Specific Disorders of Fatty Acid Oxidation

Fatty acid oxidation disorders develop in the absence or significant reduction of a specific enzyme needed in the metabolic degradation of fatty acids. Three common deficiencies of FAOD are described in detail according to the length of the acylcarnitine molecule.

### Carnitine Palmitoyltransferase I Deficiency

Carnitine palmitoyltransferase I (CPT I) deficiency is a disorder of long-chain fatty acid oxidation that is autosomal recessive. The enzyme CPT I is located on the outer membrane of the mitochondria and is vital in the transfer of long-chain fatty acids into the mitochondria (Fig 6).<sup>33-35</sup> Before long-chain fats can be catabolized, the long-chain fats are changed to their CoA esters by long-chain acyl-CoA synthetases located in the mitochondrial outer membrane. Carnitine palmitoyltransferase I catalyzes the transfer of the long-chain acyl-CoA esters to carnitine, which is transported across the mitochondrial inner membrane by carnitine acylcarnitine carrier. Once inside on the inner membrane, carnitine palmitoyltransferase II (CPT II) then reverses the CPT I reaction, reconverts the acyl-CoA in the mitochondria

matrix, which can then serve as a substrate for  $\beta$ -oxidation and releasing carnitine. In CPT I deficiency, long-chain fatty acids are not transferred from acyl-CoA esters to carnitine to acylcarnitines by CPT I.<sup>36</sup> Thus, no acylcarnitines are made and do not enter the mitochondria for subsequent  $\beta$ -oxidation. The defect leads to inadequate formation of ketone bodies in response to fasting along with inadequate gluconeogenesis and hypoglycemia. This is characterized by metabolic decompensation occurring with fasting or catabolic stress, such as a fever.<sup>35-37</sup> The classic clinical presentation may include lethargy, seizures, coma, and hepatomegaly, and can be associated with potentially fatal hypoketotic hypoglycemia.

The first description of CPT I deficiency was in 1980.<sup>38</sup> Before MS/MS, less than 50 people worldwide had been diagnosed with CPT I deficiency. In 2001, CPT deficiency was identified in dried newborn blood spots using MS/MS.<sup>39</sup> Analysis from MS/MS revealed a significantly elevated free carnitine and increased free carnitine to the sum of palmitoylcarnitine and stearyl carnitine (C0/C16 + C18). At the same time, a specific CPT I polymorphism was identified within the American and Canadian Hutterites of the Northern Plains.<sup>40</sup> This led to the development of a pilot project for a DNA based newborn screening program to better identify newborns at risk within this population.<sup>41</sup> Subsequently, a different CPT I polymorphism has been identified within the Alaska Natives, Inuit and First Nation tribes in Alaska and Canada from newborn screening with MS/MS.<sup>42-44</sup> The current treatment recommendation is simply the avoidance of fasting and the provision of caloric support during times of stress, such as diarrhea or vomiting. Carnitine supplementation is not needed because these infants have an abundance of free carnitine. The natural history of CPT I within these three groups is unknown. Further studies will be needed to determine how common the specific polymorphisms are within each of the groups and to determine the health consequences of the mutation in these populations. Additional studies will also be needed to

**Table 2. Fatty Acid Oxidation Disorders Associated with Abnormal Carnitine and Acylcarnitine Analyte Levels from Tandem Mass Spectrometry During the Neonatal Period**

Analyte	Name of Acylcarnitine	Change		Fatty Acid Oxidation Disorder to Consider
		↑	↓	
C0	Carnitine (total)	X		CPT I CUD
C2	Acetyl		X	CUD or Insufficiency
C4	Butyryl	X		SCAD, MADD
C4-OH	3-Hydroxybutyrylcarnitine	X		MCHAD, SCHAD
C5	Isovaleryl	X		GA II
C5-DC	Glutaryl	X		GA II
C6	Hexanoyl	X		GA II, MCAD
C8	Octanoyl	X		MCAD, MCAT
C8-OH	Hydroxyoctanoylcarnitine	X		MCAT
C10	Decanoyl	X		MCAD
C10-OH	Hydroxydecanoylcarnitine	X		MCAT
C10:1	Decenoyl	X		MCAD
C10:2	Decadienoylcarnitine	X		DE RED
C12	Dodecanoyl	X		GA II
C14	Tetradecanoyl	X		VLCAD
C14:1	Tetradecenoyl	X		VLCAD
C14:2	Tetradodecenoyl	X		VLCAD
C16	Palmitoyl	X		CACT, CPT II, GA II, LCHAD, VLCAD
C16-OH	3-Hydroxypalmitoyl	X	X	CPT I LCHAD, TFP
C18	Octadecanoyl	X		CPT II, VLCAD
C18:1	Linoleoyl	X	X	CPT I CACT, CPT II, GA II, LCHAD, VLCAD
C18:1-OH	3-Hydroxylinoleoyl	X	X	CPT I LCHAD, TFP

Data from Sweetman L, Millington DS, Therrel BL et al: Naming and Counting Disorders (Conditions) Included in Newborn Screening Panels. *Pediatrics*. 2006;117:S308-S314. Millington DS: Tandem mass spectrometry in clinical diagnosis, in Blau N, Duran M, Blaskovics ME, Gibson KM (eds.): *Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases*. 2nd ed. Berlin, Germany, Springer, pp 57-85,2003; Northwest Regional Newborn Screening Program: MS/MS Cutoffs, Follow up Tests. Portland, Oregon: 2005.

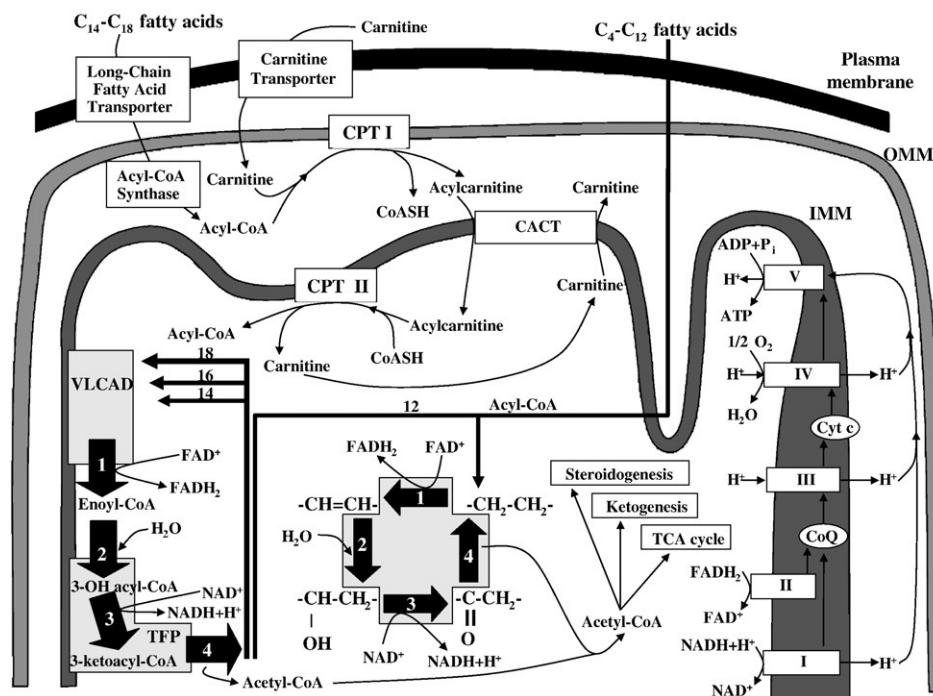
CACT = carnitine acylcarnitine translocase deficiency, CPT I = carnitine palmitoyltransferase I deficiency, CPT II = carnitine palmitoyltransferase II deficiency, CUD = carnitine uptake defect/transporter deficiency, DE RED = 2,4-dienoyl-CoA reductase deficiency, GA II = glutaric acidemia II (also known as MADD = multiple acyl CoA dehydrogenase deficiency and Ethylmalonic-adipic aciduria), LCHAD = long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency, MCAD = medium chain acyl CoA dehydrogenase deficiency, MCAT = medium chain ketoacyl-CoA thiolase deficiency, MCHAD = medium chain L-3-hydroxyacyl CoA dehydrogenase deficiency, SCAD = short-chain acyl CoA dehydrogenase deficiency, SCHAD = Short chain L-3-hydroxyacyl CoA dehydrogenase deficiency, TFP = Trifunctional protein deficiency, VLCAD = Very long chain acyl CoA dehydrogenase deficiency.

determine whether all of the affected infants are being identified via MS/MS analysis or if screening via DNA mutation analysis is required for full ascertainment.

### Medium Chain Acyl-CoA Dehydrogenase Deficiency

Medium chain acyl-CoA dehydrogenase deficiency is the most common disorder of fatty acid oxidation.<sup>45</sup> The incidence of MCAD deficiency is estimated at 1/6000 to 1/25 000 white births.<sup>46-49</sup> This makes MCAD the most common inborn error of metabolism disorder, even more common than PKU, a disorder screened for in all American states and many countries. Medium chain acyl-CoA dehydrogenase deficiency is an autosomal recessive disorder that was first described in 1982.<sup>50</sup> The gene

for MCAD is located on chromosome 1, and the nucleotide sequence of its complementary DNA has been established.<sup>51</sup> The most common mutation in people of Northern European descent is in the nucleotide 985 where A has been changed to G, leading to a lysine to glutamic acid change in the residue 329 of the protein.<sup>19,46-48</sup> Between 75% and 80% of patients are homozygous for this common mutation, whereas another 18% are heterozygous for this mutation on one allele.<sup>19,47-49,51,52</sup> No other common mutation has been identified.<sup>46,53</sup> There appears to be no clear correlation between mutation type (the genetic defect) and clinical phenotype (the physical characteristics).<sup>54</sup> The enzyme MCAD is one of the mitochondrial acyl CoA dehydrogenases that are needed to catalyze the initial steps in the  $\beta$ -oxidation of fatty acids during hepatic ketogenesis (Fig. 6). Medium chain acyl-CoA dehydrogenase involves fatty acyl-



**Fig 4.** Pathway of fatty acid metabolism. This schematic shows the transport of long-chain fatty acids and carnitine across the plasma membrane to the production of acetyl-CoA. Medium and short-chain fatty acids do not require an active transport mechanism to reach the mitochondrial matrix. Enzymes of the carnitine cycle (CPT I, CACT, and CPT II) shuttle long-chain fatty acids across the mitochondrial membranes. The fatty acid  $\beta$ -oxidation spiral includes an FAD-dependent acyl-CoA dehydrogenase step (1) followed by a 2,3 enoyl-coA hydratase reaction (2), the NAD-dependent 3-hydroxyacyl-CoA dehydrogenase step (3), and the thiolase cleavage reaction (4). Oxidation of long-chain fatty acids, which are membrane bound enzymes, and medium- and short-chain fatty acids, which are within the matrix, are shown separately. Reducing equivalents ( $\text{FADH}_2$  and  $\text{NADH}+\text{H}^+$ ) are directed to the oxidation phosphorylation pathway (right). CACT indicates carnitine acylcarnitine translocase; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; TCA, tricarboxylic acid; TFP, trifunctional protein. Figure designed by J. Joshua, Jacobson (Mayo High School Gifted and Talented Education Program, Rochester, MN.) Reprinted, with permission, from the Annual Review of Physiology, Volume 64 © 2002 by Annual Reviews [www.annualreviews.org](http://www.annualreviews.org).

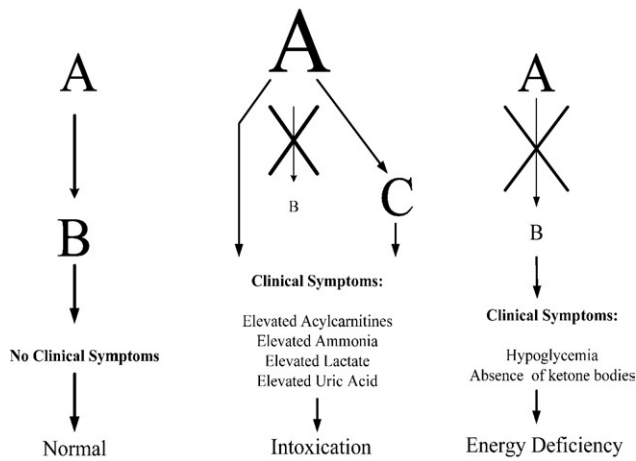
CoAs within an acid length of 6 to 12 carbons. The metabolic block in MCAD deficiency results in the inability to oxidize fatty acids to produce energy.<sup>55</sup>

The clinical presentation of MCAD deficiency during infancy can range from a severe clinical course with metabolic acidosis and hyperammonemia, hypoketotic hypoglycemia, hepatic fatty infiltrates, cardiomyopathy, or sudden infant death to a mild presentation with hypoglycemia to totally asymptomatic.<sup>56-63</sup> The classic management of MCAD deficiency has been simply the avoidance of fasting and the provision of caloric support during times of stress, such as childhood illnesses.<sup>63</sup> The prognosis is excellent when the diagnosis is established and frequent feedings are instituted to avoid prolonged fasting.<sup>58</sup> Supplementation with carnitine is controversial. Oral carnitine supplementation ranges from 50 to 100 mg/kg per day of carnitine to correct the secondary carnitine deficiency and to enhance the elimination of toxic metabolites.<sup>48</sup> Initial studies

with oral carnitine supplementation reported an increase in the conjugation and subsequent urinary excretion of acyl groups.<sup>64,65</sup> There was an increase in the availability of free CoA and decreased acyl-CoA accumulation in the mitochondria. Other studies revealed findings that cast doubt on the value of long-term treatment with carnitine in patients with MCAD.<sup>66</sup> The supplementation of carnitine inhibited glycine conjugation, which is the major pathway for the disposal of C6 to C8 acylcarnitine analytes. No randomized double-blind study has been done to answer the question if supplementation with carnitine is needed in patients with MCAD.

### Very Long-Chain Acyl-CoA Dehydrogenase Deficiency

Very long-chain acyl CoA dehydrogenase (VLCAD) deficiency is the most common mitochondrial  $\beta$ -oxidation defect of



**Fig 5.** Impaired fatty acid metabolism of a generic enzyme reaction. A is the substrate. B is the product. C is a product of an alternative pathway. Adapted from P. Rinaldo, Matern D: Disorders of fatty acid transport and mitochondrial oxidation. *Genet Med.* 2 (2000) 338-344 with permission from Lippincott Williams & Wilkins.

long-chain fatty acids.<sup>67</sup> However, the incidence is rare, approximately 1:50 000 to 1:100 000 births.<sup>68</sup> Originally described in the early 1990s, VLCAD deficiency is an autosomal recessive genetic disorder that results in the inability to oxidize long-chain fatty acids to produce energy (Fig. 6) that is needed by highly oxidative organs, such as the heart.<sup>69-71</sup> Very long-chain acyl CoA dehydrogenase is an enzyme within the mitochondrial matrix and catalyses the first step in the  $\beta$ -oxidation spiral of long-chain fatty acids.<sup>72,73</sup> Very long-chain acyl CoA dehydrogenase involves fatty acyl CoAs in the acid length of 14 to 20 carbons length. This is not much larger than the 12- to 18-carbon range of the mitochondrial matrix enzyme, long-chain acyl CoA dehydrogenase (LCAD). As a result, several patients originally identified as having LCAD deficiency in reality had VLCAD deficiency.<sup>74</sup> One case of VLCAD deficiency reported was identified retrospectively to determine if the case would have been identified by MS/MS on the newborn's original newborn screening card.<sup>75</sup> The acylcarnitine profile from the newborn's original newborn screening card was analyzed 2 years after it was obtained. There was a significant accumulation of long-chain acylcarnitines with a significant peak of tetradecenoylcarnitine (C14:1), the identified marker of VLCAD deficiency. Subsequently, through newborn screening using MS/MS, eight asymptomatic infants were identified.<sup>67</sup> Biochemical testing confirmed VLCAD in all cases.

The clinical presentation of VLCAD deficiency during infancy can range from a severe clinical course with metabolic acidosis, hypoketotic hypoglycemia, massive hepatic fatty infiltrates, hypertrophic cardiomyopathy, or sudden death to a mild presentation of hypoglycemia to totally asymptomatic.<sup>68,73-80</sup> Several studies have demonstrated a clear genotype-phenotype relationship between the mutation with clinical phenotype.<sup>68,81-83</sup> Infants with severe clinical

presentations have mutations that result in no residual enzyme activity, whereas infants with milder presentations have mutations that result in residual enzyme activity. This is in stark contrast to what has been observed in MCAD deficiency, in which no correlation between genotype and phenotype can be established.<sup>51,54,84,85</sup>

The management of VLCAD deficiency has included a high-carbohydrate low long-chain fat diet with medium chain triglyceride supplementation, the avoidance of fasting, and the prompt intervention with parenteral glucose solutions when fasting is unavoidable or due to stress as with vomiting or fever.<sup>85,86</sup> Dietary management with asymptomatic infants varies from implementation of the classic diet to the continuation of full breastfeeding with diet modification after transition to solids with close monitoring of complications.<sup>68</sup> In addition, carnitine supplement is controversial.<sup>76,87</sup> Recent research has revealed that low carnitine concentrations in plasma associated with increased production and accumulation of long-chain acylcarnitines do not reflect carnitine homeostasis in tissues.<sup>67</sup> Long-term follow-up of infants with VLCAD is needed to determine outcomes and to define the risk of future complications and appropriate treatment options.

## Implications for Clinical Care

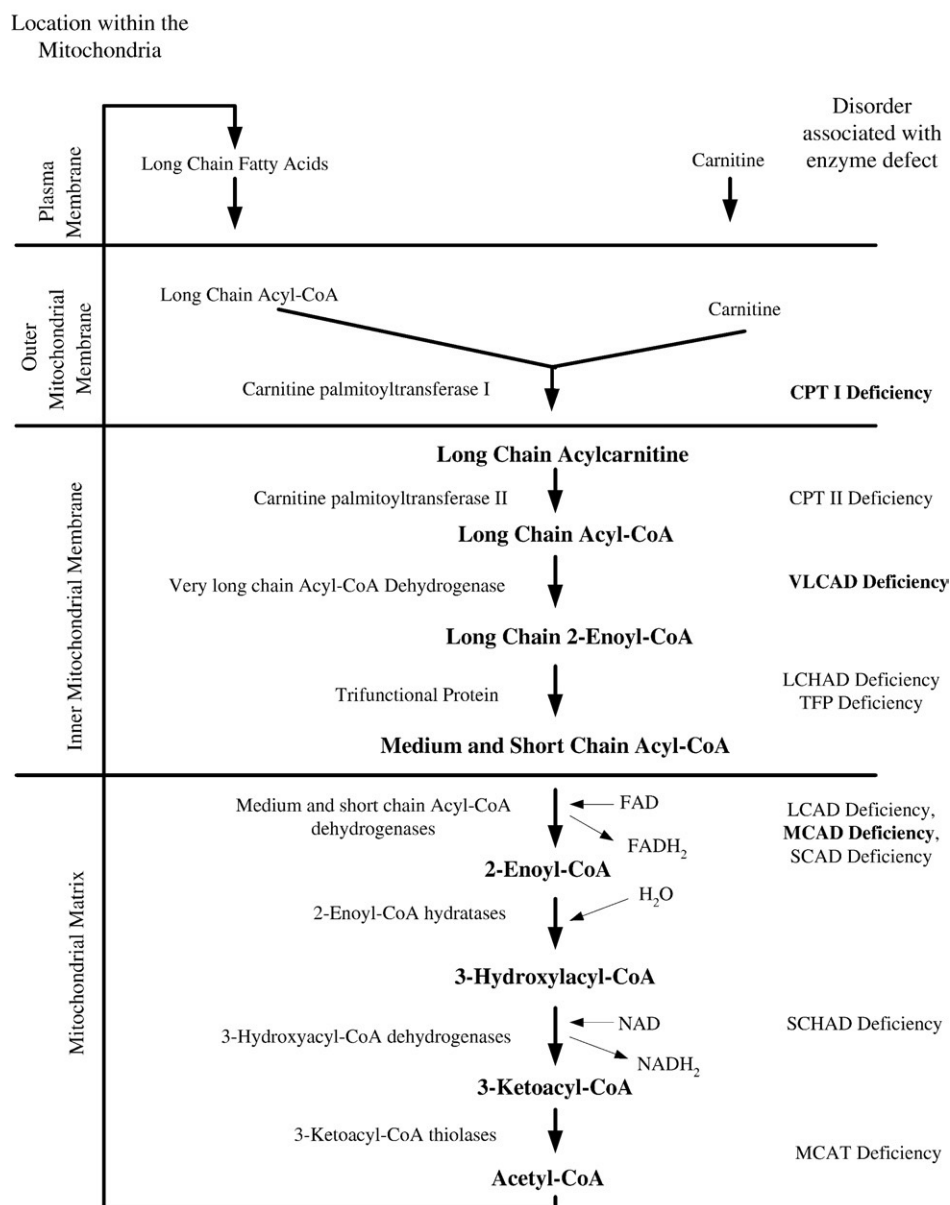
Families need to be educated regarding the importance of newborn screening and the specific disorders that are screened for in the state where their infant is born. Not all states and territories screen for FAOD using MS/MS. As of July 2007, 46 states use MS/MS for newborn screening.<sup>88</sup> Within this group, the FAOD screened for varies. In addition, results of the newborn screen are usually not available at the time of discharge from the normal newborn nursery. Parents should be encouraged to ask their primary care provider about the results at their first follow-up clinic visit. Early diagnosis improves outcome. Most of the therapies are lifelong and requires continual nutritional, medical, and laboratory monitoring. As infants are surviving into childhood and adulthood, their long-term physical, mental, and emotional needs are becoming increasingly important. As these children reach adulthood, their reproductive abilities and the teratogenic effect that their disease or its treatment can have on their children are unknown.

## Summary

With MS/MS, the world of newborn screening has changed from the "one test-one disorder" system to "one test-many disorders." Disorders of fatty acid oxidation can be rapidly identified with a dried newborn blood spot on filter paper that is used to detect many other inborn error of metabolism. MS/MS allows the opportunity to identify many newborns with a FAOD before a catastrophic insult, thus, decreasing morbidity and mortality. In addition, MS/MS allows improved understanding of the natural history of FAODs, such as CPT I, MCAD, and VLCAD deficiencies. With appropriate support for biochemical



## Fatty Acid Oxidation Pathway



**Fig 6.** Simplified diagram of the fatty acid oxidation pathway with location of enzyme within the mitochondria and identification of possible FAOD when enzyme reaction is impaired. MCAT deficiency indicates medium chain ketoacyl-CoA thiolase deficiency; SCAD deficiency, short-chain acyl CoA dehydrogenase deficiency; SCHAD deficiency, short-chain L-3-hydroxyacyl CoA dehydrogenase deficiency; TFP deficiency, trifunctional protein deficiency.

genetics expertise, MS/MS used in newborn screening programs can provide a positive impact on the health of infants with a FAOD and their families.

## References

- Guthrie R, Susi A. A simple phenylalanine method for detecting PKU in large, populations of newborn infants. *Pediatrics*. 1963;32:338-343.
- Beasley M, Costello P, Smith I. Outcome of treatment in young adults with phenylketonuria detected by routine neonatal screening between 1964 and 1971. *Q J Med*. 1994; 87:155-160.
- MacGready RA, Hussey MG. Newborn phenylketonuria detection program in Massachusetts. *Am J Public Health*. 1964;54:2075.
- National Research Council, Committee for the study of inborn errors of metabolism. Genetic screening: programs,

- principles, and research. Washington, DC: National Academy of Science; 1975.
5. Newborn Screening Task Force. Serving the family from birth to the medical, home: a report from the newborn screening task force. *Pediatrics*. 2000;106: S383-S427.
  6. Paul DB. The history of phenylketonuria screening in the US. In: Holtzman NA, Watson MS, editors. Promoting safe and effective genetic testing in the United States: final report of the Task Force on Genetic Testing. Bethesda (MD): National Institute of Health; 1997.
  7. President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Screening and counseling for genetic conditions: a report on the ethical, social and legal implications of genetic screening and counseling, and education programs. Washington DC: Government Printing Office; 1983.
  8. Wilson JMG, Jungner F. Principles and practice of screening for disease (Public Health Paper #34). Geneva: World Health Organization; 1968.
  9. American Academy of Pediatrics. Issues in newborn screening. *Pediatrics*. 1992;89:135-146.
  10. Therrell BL, Panny SR, Davidson A, et al. U.S. newborn screening system, guidelines: statement of the Council of Regional Networks for Genetic Services. *Screening*. 1992;1: 135-147.
  11. Comeau AM, Hsu HW, Schwerzlw M, et al. Detection of HIV in specimens from newborn screening programs. *N Engl J Med*. 1992;326:1703.
  12. Schoen EJ, Black S, Cohen D, et al. Screening for neonatal toxoplasmosis. *N Engl J Med*. 1994;331:1456-1458.
  13. Postellon DC. Screening for congenital hypothyroidism. *J Pediatr*. 1981;9:170-171.
  14. Levy HL, Hammersen G. Newborn screening for galactosemia and other galactose metabolic defects. *J Pediatr*. 1978; 92:871-877.
  15. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. *J Inherit Metab Dis*. 1990;13:802-818.
  16. Millington DS, Roe CR, Maltby DA. Application of high resolution fast atom bombardment and constant B/E ratio linked scanning to the identification and analysis of acylcarnitines in metabolic disease. *Biomed Mass Spectrom*. 1984;11:236-241.
  17. Millington DS, Kodo N, Norwood DL, Roe CR. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. *J Inherit Metab Dis*. 1990;13: 321-324.
  18. Millington DS, Koeberl D. Metabolic screening in the newborn. *Growth Genet Horm*. 2003;19:33-38.
  19. Zytovicz TH, Fitzgerald EF, Marsden D, et al. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two year summary from the New England Newborn Screening Program. *Clin Chem*. 2001;47:1945-1955.
  20. Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Semin Perinatol*. 1999;23:183-193.
  21. Millington DS, Terade N, Chace DH, et al. The role of tandem mass spectrometry in the diagnosis of fatty acid oxidation disorders. In: Coates PM, Tanaka K, editors. New development in fatty acid oxidation: proceedings of the second International Symposium on clinical, biochemical, and molecular aspects of fatty acid oxidation. New York: Wiley-Liss; 1991. p. 339-354.
  22. Chace DH, Millington DS, Terada N, et al. Rapid diagnosis of phenylketonuria by quantitative analysis for phenylalanine and tyrosine in neonatal blood spots by tandem mass spectrometry. *Clin Chem*. 1993;39: 66-71.
  23. Schmidt-Sommerfeld E, Penn D, Duran M, et al. Detection of inborn errors of fatty acid oxidation from acylcarnitine analysis of plasma and blood spots with radioisotopic exchange-high-performance liquid chromatographic method. *J Pediatr*. 1993;122:708-714.
  24. Van Hove JL, Zhang W, Kahler SG, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: diagnosis by acylcarnitine analysis in blood. *Am J Hum Genet*. 1993;52: 958-966.
  25. Chace DH, Hillman SL, Millington DS, et al. Rapid diagnosis of maple syrup urine disease in blood spots from newborns by tandem mass spectrometry. *Clin Chem*. 1995;41:62-68.
  26. Millington DS. Tandem mass spectrometry in clinical diagnosis. In: Blau N, Duran M, Blaskovics ME, Gibson KM, editors. Physician's guide to the laboratory Diagnosis of metabolic diseases. 2nd ed. Berlin, Germany: Springer; 2003. p. 57-85.
  27. Sewell AC, Bohles HJ. Acylcarnitines in intermediary metabolism. *Eur J Pediatr*. 1995;154:871-877.
  28. Carter AL, Abney TO, Lapp DF. Biosynthesis and metabolism of carnitine. *J Child Neurol*. 1995;10(Suppl 2): S3-S7.
  29. Chace DH, Hillman SL, Van Hove JL, et al. Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry. *Clin Chem*. 1997;43: 2108-2113.
  30. Ziadeh R, Hoffman EP, Finegold DN, et al. Medium chain acyl-CoA dehydrogenase deficiency in Pennsylvania: neonatal screening shows high incidence and unexpected mutation frequencies. *Pediatr Res*. 1995;37: 675-678.
  31. American College of Medical Genetics. Standardization of outcomes and guidelines for state newborn screening program. *Fed Regist*. 2005;70 Department of Health and Human Services. Available at: [www.mchc.hrsa.gov/screening/summary.htm](http://www.mchc.hrsa.gov/screening/summary.htm). [Retrieved July 30, 2007].
  32. Bennett MJ. The enzymes of mitochondrial fatty acid oxidation. *Clin Chim Acta*. 1994;226:213-224.
  33. Krebs HA. The history of the tricarboxylic acid cycle. *Perspect Biol Med*. 1970;14:154-170.

34. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem.* 1997;244:1-14.
35. Brown NF, Mullur RS, Subramanian I, et al. Molecular characterization of L-CPT I deficiency in six patients: insight into function of the native enzyme. *J Lipid Res.* 2001;42:1134-1142.
36. Bougneres PF, Saudubray JM, Marsac C, et al. Fasting hypoglycemia resulting from hepatic carnitine palmitoyltransferase deficiency. *J Pediatr.* 1981;98:742-746.
37. Bonnefont JP, Haas R, Wolff R, et al. Deficiency of carnitine palmitoyltransferase I. *J Child Neurol.* 1989;4:198-203.
38. Bougneres PF, Saudubray JM, Marsac C, et al. Decreased ketogenesis due to deficiency of hepatic carnitine acyltransferase. *N Engl J Med.* 1980;302:123-124.
39. Fingernut R, Roschinger W, Muntau AC, et al. Hepatic palmitoyltransferase I deficiency: acylcarnitine profiles in blood spots are highly specific. *Clin Chem.* 2001;47:1763-1768.
40. Prip-Buus C, Thuiller L, Abadi N, et al. Molecular and enzymatic characterization of a unique carnitine palmitoyltransferase 1A mutation in the Hutterite community. *Mol Genet Metab.* 2001;73:46-54.
41. Prasad C, Johnson JP, Bonnefont JP, et al. Hepatic carnitine palmitoyltransferase 1 (CPT1 A) deficiency in North American Hutterites (Canadian and American): evidence for a founder effect and results of a pilot study on a DNA based newborn screening program. *Mol Genet Metab.* 2001;73:55-63.
42. Seargeant LE, Stier A, Prasad C, et al. Preliminary evidence for high frequency of combined CPT 1 and CPT 2 mutations in the Canadian Inuit. In: IX International Congress on Inborn Errors of Metabolism, Brisbane, Australia, September 2-6, 2003. *J Inherit Metab Dis.* 2003;26(suppl 2):197.
43. Sinclair G, Waters PJ, Vallance H, et al. The dilemma of the CPT-1 P479L mutation in Canadian Inuit and First Nations Families. 6th International Congress on Fatty Acid Oxidation, Egmond aan Zee, the Netherlands, 21, 2005; 2005.
44. Gillingham MB, Banta-Wright SA, Bennett MJ, et al. High incidence of the carnitine palmitoyltransferase-1A P479L variant identified in Alaska native infants by expanded newborn screening. In preparation.
45. Roe CR, Ding J. Mitochondrial fatty acid oxidation disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic and molecular bases of inherited disease.* 9th ed. New York: McGraw-Hill; 2001. p. 2297-2326.
46. Maier EM, Leibl B, Roschinger W, et al. Population spectrum of ACADM genotypes correlated to biochemical phenotypes in newborn screening for medium-chain acyl-CoA dehydrogenase deficiency. *Hum Mutat.* 2005;25:443-452.
47. Chace DH, Kalas TA, Naylor EW. The application of tandem mass spectrometry in neonatal screening for inherited disorders of intermediary metabolism. *Annu Rev Genomics Hum Genet.* 2002;3:17-45.
48. Carpenter K, Wiley V, Sim KG, et al. Evaluation of newborn screening for medium chain acyl-CoA dehydrogenase deficiency in 275,000 babies. *Arch Dis Child Fetal Neonatal Ed.* 2001;85:F105-F109.
49. Sanders S, Janze N, Janetzky B, et al. Neonatal screening for medium acyl-CoA deficiency: high incidence in lower Saxony (northern Germany). *Eur J Pediatr.* 2001;160:318-319.
50. Kolvraa S, Gregersen N, Christensen E, Hobolth N. In vitro fibroblast studies in a patient with C<sub>6</sub>-C<sub>10</sub> dicarboxylic aciduria: evidence for a defect in general acyl-CoA dehydrogenase. *Clin Chim Acta.* 1982;126:53-67.
51. Kidd JR, Matsubara Y, Castiglione CM, et al. The locus for the medium-chain acyl-coA dehydrogenase gene on chromosome 1 is highly polymorphic. *Genomics.* 1990;6:89-93.
52. Andresen BS, Sobrowolski SF, O'Reilly L, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Am J Hum Genet.* 2001;68:1408-1418.
53. Tajima G, Sakura N, Ono H, et al. Medium chain acyl-coA dehydrogenase deficiency found through newborn screening by tandem mass spectrometry in Japan. *J Inherit Metab Dis.* 2004(Suppl 1):11.
54. Andresen BS, Bross P, Udvari S, et al. The molecular basis for medium-chain acyl-coA dehydrogenase (MCAD) deficiency in compound heterozygous patients: is there correlation between genotype and phenotype? *Hum Mol Genet.* 1997;6:695-707.
55. Coates PM, Hale DE, Stanley CA, et al. Genetic deficiency of medium-chain acyl coenzyme a dehydrogenase: studies in cultured skin fibroblasts and peripheral mononuclear leukocytes. *Pediatr Res.* 1985;19:671-676.
56. Bennett MJ, Rinaldo P, Millington DS, et al. Medium chain acyl-coA dehydrogenase deficiency: postmortem diagnosis in case of sudden infant death and neonatal diagnosis of an affected sibling. *Pediatr Pathol.* 1991;11:889-895.
57. Rashad MS, Ozand PT, Bennett MJ, et al. Inborn errors of metabolism diagnosed in sudden death cases by acylcarnitine analysis of postmortem bile. *Clin Chem.* 1995;41(8 pt1):109-111.
58. Iafolla AK, Thompson RJ, Roe CR. Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. *J Pediatr.* 1994;124:409-415.
59. Christodoulou J, Hoare J, Hammond J, et al. Neonatal onset of medium-chain acyl-coenzyme A dehydrogenase deficiency with confusing biochemical features. *J Pediatr.* 1995;126:65-68.
60. Wilcox RL, Nelson CC, Stenzel P, Steiner RD. Post-mortem screening for fatty acid oxidation disorders by analysis of Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *J Pediatr.* 2002;141:833-836.

61. Emery JL, Variend S, Howat AJ, et al. Investigation of inborn errors of metabolism in unexpected infant deaths. *Lancet*. 1988;2:29-31.
62. Harpey JP, Charpentier C, Paturneau-Jouas M. Sudden infant death syndrome and inherited disorders of fatty acid *b*-oxidation. *Biol Neonate*. 1990;58(Suppl 1):70-80.
63. Treem WR, Stanley CA, Goodman SI. Medium-chain acyl-CoA dehydrogenase deficiency: metabolic effects and therapeutic efficacy of long-term L-carnitine supplementation. *J Inherit Metab Dis*. 1989;12:112-119.
64. Stumpf DA, Parker WD, Angelini C. Carnitine deficiency, organic acidemias and Reye's syndrome. *Neurology*. 1985;35:1041-1045.
65. Roe CR, Millington DS, Kahler SG, et al. Carnitine homeostasis in the organic acidurias. In: Tanaka K, Coates PM, editors. *Fatty acid oxidation: clinical, biochemical and molecular aspects*. New York: Alan R. Liss; 1990. p. 382-402.
66. Rinaldo R, Schmidt-Sommerfeld E, Posca AP, et al. Effect of treatment with glycine and L-carnitine in medium-chain acyl-coenzyme a dehydrogenase deficiency. *J Pediatr*. 1993;122:580-584.
67. Spieketkoetter U, Tokunaga C, Wendel U, et al. Tissue carnitine homeostasis in very-long-chain acyl-CoA dehydrogenase-deficient mice. *Ped Res*. 2005;57:760-764.
68. Spiekerkoetter U, Sun B, Zytkovicz T, et al. MS/MS-based newborn and family screening detects asymptomatic patients with very-long-chain acyl-CoA dehydrogenase deficiency. *J Peds*. 2003;143:335-342.
69. Bertran C, Largilliere C, Zabot MT, et al. Very-long-chain acyl-coA dehydrogenase deficiency: identification of a new inborn error of mitochondrial fatty acid oxidation in fibroblasts. *Biochim Biophys Acta*. 1993;1180:327-329.
70. Aoyama T, Uchida Y, Kelley RI, et al. A novel disease with deficiency of mitochondrial very-long-chain acyl-CoA dehydrogenase. *Biochem Biophys Res Commun*. 1993;191:1369-1372.
71. Iwai K, Uchida Y, Orii T, Hashimoto. Novel fatty acid beta-oxidation enzymes in rat liver mitochondria. I. Purification and properties of very-long-chain acyl-coenzyme A dehydrogenase. *J Biol Chem*. 1992;267:1027-1033.
72. Aoyama T, Ueno I, Kamijo T, Hashimoto T. Rat very-long-chain acyl-CoA dehydrogenase, a novel mitochondrial acyl-CoA dehydrogenase gene product, is a rate-limiting enzyme in long-chain fatty acid beta-oxidation system. cDNA and deduced amino acid sequence and distinct specificities of the cDNA-expressed protein. *J Biol Chem*. 1994;269:19088-19094.
73. Hale DE, Bennett MJ. Fatty acid oxidation disorders: a new class of metabolic diseases. *J Pediatr*. 1992;121:1-11.
74. Yamaguchi S, Idno Y, Coates PM, et al. Identification of very-long-chain acyl-Coa dehydrogenase deficiency in three patients previously diagnosed with long-chain acyl-CoA dehydrogenase deficiency. *Ped Res*. 1993;34:111-113.
75. Wood JC, Magera MJ, Rinaldo P, et al. Diagnosis of very long chain acyl-dehydrogenase deficiency from an infant's newborn screening card. *Pediatrics*. 2001;108:E19.
76. Touma EH, Rashed MS, Vianey-Saban C, et al. A severe genotype with favourable outcome in very long chain acyl-coA dehydrogenase deficiency. *Arch Dis Child*. 2001;84:58-60.
77. Yoon N, Strauss AW, Yoo HW. Sudden death in a Korean infant with very-long-chain acyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis*. 2001;24:407-408.
78. Bonnett D, Martin D, de Lonlay P, et al. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation*. 1999;100:2248-2253.
79. Hahn SH, Lee EH, Jung JW, et al. Very long chain acyl coenzyme A dehydrogenase deficiency in a 5-month-old Korean boy: identification of a novel mutation. *J Peds*. 1999;135:250-253.
80. Boles RG. Very long-chain acyl-CoA dehydrogenase deficiency in an infant presenting with massive hepatomegaly. *J Inherit Metab Dis*. 2002;25:315-316.
81. Gregersen N, Andresen BS, Corydon MJ, et al. Mutation analysis in mitochondrial fatty acid oxidation defects: exemplified by acyl-CoA dehydrogenase deficiencies, with special focus on genotype-phenotype relationship. *Human Mutat*. 2001;12:169-189.
82. Cox KL, Hamm DA, Millington DS, et al. 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*. 2001;10:2069-2077.
83. Andresen BS, Olpin S, Poorthuis BJHM, et al. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. *Am J Hum Genet*. 1999;64:479-494.
84. Andresen BS, Dobrowolski SF, O'Reilly L, et al. Medium-chain acyl-CoA dehydrogenases (MCAD) mutations identified by MS/MS based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Am J Hum Genet*. 2001;68:1408-1418.
85. Gregersen N, Andresen BS, Bross P, et al. Molecular characterization of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: identification of a lys329 to glu mutation in the MCAD gene and expression of inactive mutant protein in E coli. *Hum Genet*. 1991;86:545-551.
86. Cox GF, Souiri M, Aoyama T, et al. Reversal of severe hypertrophic cardiomyopathy and an excellent neurophysiologic outcome in very-long-chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr*. 1998;133:247-253.
87. Spiekerkotter U, Schwahn B, Korall H, et al. Very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency: monitoring of treatment by carnitine/acylcarni-

tine analysis in blood spots. *Acta Paediatr.* 2000;89: 492-495.

88. National Newborn Screening and Genetic Resources Center (NNSGRC). Available at: <http://genes-r-us.uthscsa.edu/resources/newborn/screenstatus.htm>. [Retrieved July 30, 2007].

### Further Reading

1. <http://www.fodsupport.org/>: Fatty oxidation disorders communication network.
2. <http://www.simd.org/>: Society for Inherited Metabolic Disorders (USA).

3. <http://www.ssiem.org.uk/ssiemj.html>: Society for the Study of Inborn Errors of Metabolism (UK).

4. <http://www.franken.de/lists/metab-l/>: Inborn errors of metabolism list for professionals.

5. <http://www.tylerforlife.com>: Tyler for Life Foundation (newborn screening).

6. <http://genes-r-us.uthscsa.edu>: National Newborn Screening and Genetics Resource Center.

7. <http://www.rarediseases.org/cgi-bin/nord>: National Organization for Rare Disorders.

8. <http://www.geneclinics.org>: GeneClinics: Medical Genetics Knowledge Base at University of Washington, Seattle, Washington.