2008

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

Sandra A. Banta-Wright  
*George Fox University, sbantawright@georgefox.edu*

Kathleen C. Shelton

Michael J. Bennett

Follow this and additional works at: [http://digitalcommons.georgefox.edu/sn_fac](http://digitalcommons.georgefox.edu/sn_fac)  
Part of the [Maternal, Child Health and Neonatal Nursing Commons](http://digitalcommons.georgefox.edu/sn_fac)

**Recommended Citation**  
Published in Newborn and Infant Nursing Reviews, 8(1), March 2008
Disorders of Fatty Acid Oxidation in the Era of Tandem Mass Spectrometry in Newborn Screening

Sandra A. Banta-Wright, RNC, MN, NNP, Kathleen C. Shelton, PhD, PNP, and Michael J. Bennett, PhD, FRCPath, FACB

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

In 1961, Guthrie and Susi 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. By 1961, Oregon and Massachusetts began screening every newborn in those states for PKU. 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. 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. 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. 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. In other cases, the benefits of medical intervention were less dramatic but significant enough to warrant screening.

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. 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).

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

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).\textsuperscript{26} 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.\textsuperscript{18,20} 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.\textsuperscript{27} 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.\textsuperscript{28} 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.\textsuperscript{24,27,29,30} In 2005, a consensus statement was issued to screen without further delay at least for MCAD deficiency in all 50 states.\textsuperscript{31}

**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).\textsuperscript{32,33}
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 1 Deficiency

Carnitine palmitoyltransferase 1 (CPT 1) deficiency is a disorder of long-chain fatty acid oxidation that is autosomal recessive. The enzyme CPT 1 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). 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 1 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, reconvert the acyl-CoA in the mitochondrial matrix, which can then serve as a substrate for \( \beta \)-oxidation and releasing carnitine. In CPT 1 deficiency, long-chain fatty acids are not transferred from acyl-CoA esters to carnitine to acylcarnitines by CPT 1. Thus, no acylcarnitines are made and do not enter the mitochondria for subsequent \( \beta \)-oxidation. The defect leads to inadequate formation of ketones 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. 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 1 deficiency was in 1980. Before MS/MS, less than 50 people worldwide had been diagnosed with CPT 1 deficiency. In 2001, CPT deficiency was identified in dried newborn blood spots using MS/MS. Analysis from MS/MS revealed a significantly elevated free carnitine and increased free carnitine to the sum of palmitoylcarnitine and stearoylcarnitine (C0/C16 + C18). At the same time, a specific CPT I polymorphism was identified within the American and Canadian Hutterites of the Northern Plains. 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. 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. The natural history of CPT 1 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...
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. The incidence of MCAD deficiency is estimated at 1/6000 to 1/25,000 white births. 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. The gene for MCAD is located on chromosome 1, and the nucleotide sequence of its complementary DNA has been established. 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. Between 75% and 80% of patients are homozygous for this common mutation, whereas another 18% are heterozygous for this mutation on one allele. No other common mutation has been identified. There appears to be no clear correlation between mutation type (the genetic defect) and clinical phenotype (the physical characteristics). The enzyme MCAD is one of the mitochondrial acyl CoA dehydrogenases that are needed to catalyze the initial steps in the β-oxidation of fatty acids during hepatic ketogenesis (Fig. 6). Medium chain acyl-CoA dehydrogenase involves fatty acyl-

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

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


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.
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. 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. 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. The prognosis is excellent when the diagnosis is established and frequent feedings are instituted to avoid prolonged fasting. 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. Initial studies with oral carnitine supplementation reported an increase in the conjugation and subsequent urinary excretion of acyl groups. 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. 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 fatty acids. 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 (FADH$_2$ and NADH+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.
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.88 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 1, MCAD, and VLCAD deficiencies. With appropriate support for biochemical

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.67 However, the incidence is rare, approximately 1:50,000 to 1:100,000 births.68 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.69-71 Very long-chain acyl CoA dehydrogenase is an enzyme within the mitochondrial matrix and catalyses the first step in the β-oxidation spiral of long-chain fatty acids.72,73 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.74 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.75 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 tetradecanoicarnitine (C14:1), the identified marker of VLCAD deficiency. Subsequently, through newborn screening using MS/MS, eight asymptomatic infants were identified.67 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.68,73,80 Several studies have demonstrated a clear genotype-phenotype relationship between the mutation with clinical phenotype.68,81-83 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.51,54,84,85

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.85,86 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.85 In addition, carnitine supplement is controversial.76,87 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.67 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.

Fig 6. Biochemical pathway of fatty acid oxidation. The acylcarnitine profile from the newborn’s original newborn screening card. The identified marker of long-chain acylcarnitines with a significant peak of tetradecanoicarnitine (C14:1), the identified marker of VLCAD deficiency. Biochemical testing confirmed VLCAD in all cases.
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**


4. National Research Council, Committee for the study of inborn errors of metabolism. Genetic screening: programs,


Further Reading