Tandem Mass Spectrometry (MSMS): Testing Starts January 2006

FATTY ACID OXIDATION DISORDERS
ORGANIC ACID DISORDERS
AMINO ACID DISORDERS

Lab Processes
Methods
Interpretation
Metabolic Disorders Identified by Expanded Newborn Screening

By Nicola Longo, MD, PhD

INTRODUCTION
In January, 2006, Utah will start screening newborns for an increased number of disorders. These include a number of inborn errors of metabolism, biotinidase deficiency, and congenital adrenal hyperplasia. The new inborn errors of metabolism will be in addition to phenylketonuria and galactosemia that have been screened for several years. A new technology, tandem mass spectrometry, will be used to screen for additional inborn errors of metabolism. Two main classes of chemicals will be measured by this technique: acylcarnitines and amino acids.

Disorders identified by abnormal acylcarnitines include disorders of fatty acid oxidation and organic acidademias. The abnormal organic acids formed as the result of the deficiency can present shortly after birth with refusal of feeding, vomiting, and lethargy progressing to coma. Laboratory evaluation can reveal metabolic acidosis (with low plasma bicarbonate) and hyperammonemia.

Disorders of amino acid metabolism. Amino acids are not only the building blocks of proteins, but also serve as neurotransmitters (glutamine, glutamate, g-aminobutyric acid) or as precursors of hormones, coenzymes, pigments, purines, or pyrimidines. Eight amino acids, referred to as essential, cannot be synthesized by humans and must be obtained from dietary sources. The others are formed endogenously. Each amino acid has a unique degrading pathway by which its nitrogen and carbon components are used for the synthesis of other amino acids, carbohydrates, and lipids. Disorders of amino acid metabolism are individually rare, but collectively they affect perhaps 1 in 8,000 newborns. Almost all are transmitted as autosomal recessive traits.

In general, these disorders are named for the compound that accumulates to highest concentration in blood (-emias) or urine (-urias). For many conditions (often called aminoacidopathies), the parent amino acid is found in excess; for others, generally referred to as organic acidademias, products in the catabolic pathway accumulate. Which compound(s) accumulates depends on the site of the enzymatic block, the reversibility of the reactions proximal to the lesion, and the availability of alternative pathways of metabolic "runoff." The manifestations of these conditions differ widely from no clinical consequences to neonatal mortality. Central nervous system (CNS) dysfunction, in the form of developmental retardation, seizures, alterations in sensorium, or behavioral disturbances, is present in more than half the disorders. Protein-

"The purpose of newborn screening is to identify infants at risk and in need of more definitive testing. As with any laboratory test, both false negative and false positive results are possible. Screening test results are insufficient information on which to base diagnoses or treatment."

metabolic imbalance are conjugated with carnitine in the mitochondrial matrix, released in the cytoplasm and in the bloodstream, producing abnormal acylcarnitines in blood and plasma. This conjugation process usually aids in the removal of toxic metabolites and provides a biochemical marker to identify these disorders. All disorders identified by an abnormal acylcarnitine profile are autosomal recessive, with an incidence rate of 1:10,000-200,000 newborns. Two main groups of disorders are identified by abnormal acylcarnitines: disorders of fatty acid oxidation and organic acidademias.

Disorders of fatty acid oxidation. Fatty acids are utilized within mitochondria to produce energy. Carnitine and the carnitine cycle are required to transfer fatty acids into the mitochondria for subsequent beta-oxidation. In beta-oxidation, long-chain fatty acids are progressively shortened by two carbon units at each cycle to generate acetyl CoA that is used by the Krebs cycle to produce energy. Defects of fatty acid oxidation, such as MCAD (Medium Chain Acyl CoA Dehydrogenase) deficiency, are usually silent and become evident only when the body needs energy from fat at times of fasting, infections, or fever. Apparently healthy children become acutely sick, can lose consciousness, become comatose and die from the very first episode. (During episodes, laboratory testing can indicate hypoglycemia and increase in liver function tests.) Other fatty acid oxidation disorders (LCHAD deficiency) can also affect the muscle and the heart with pain and cardiomyopathy.

Organic Acidademias. Organic acids are chemicals produced in the intermediate metabolism of amino acids, nucleotides, and fatty acids. Organic acidademias are all autosomal recessive and the affected infant can be the first within a family. Disorders such as propionic acidemia, methylmalonic acidemia, isovaleric acidemia, .
Therapy for organic acid disorders and amino acidopathies consists in special diets restricting the compounds (usually amino acids) that result in the formation of the abnormal organic acid or the accumulation of high levels of amino acids, supplementation with vitamins specific for each disorder, carnitine supplements, and sometimes fasting avoidance. For some of these conditions, aggressive therapy of infections with IV fluids containing glucose is essential to avoid catabolism and trigger aggravation of clinical symptoms.

Screening Practice Considerations

For organic acidemias or amino acidopathies, a positive screening test may depend upon protein ingestion. Specimens obtained before 24 hours of age should be repeated. A second screening test obtained after 7 days of age is the preferred specimen to exclude primary carnitine deficiency, tyrosinemia type I, arginase deficiency, homocystinuria, carnitine palmityl transferase I deficiency. Repeated screening, between 7-21 days of age, should be obtained in all children. For fatty acid oxidation defects, the stress of birth is usually adequate to cause acylcarnitine elevation especially if the specimen is collected less than 24 hours from birth. A positive screen strongly suggestive of a fatty acid oxidation, such as VLCAD (very long chain acyl CoA Dehydrogenase) deficiency, defect might require direct enzyme assay even if confirmatory testing obtained at a later time (i.e. when the child is more metabolically compensated) is normal. Consultation with a center familiar with these disorders is critical in determining the best procedure to follow on a case-by-case basis.

For the severity of the illnesses caused by certain disorders, prompt confirmatory testing is required in some cases even if there is evidence to suggest that one of the situations associated with false positive screens is present (these include early specimen collection, prematurity, heat-damaged specimen, hyperalimentation, or antibiotic therapy). The presence of any of these conditions associated with false-positive results does not exclude the possibility of disease.

REFERENCES


Screening for metabolic disorders in newborns began over 30 years ago with assays of phenylalanine on dried blood spots to identify infants with phenylketonuria (PKU). Technology, instrumentation, and clinical advances over the past several years have led to newborn screening using tandem mass spectrometry (Chace, Kalas et al. 2003) (MSMS or TMS). The advantage of MSMS as compared to traditional screening techniques is that multiple metabolites can be detected simultaneously with one analysis from one blood spot, allowing the identification of several metabolic disorders at once. Two main classes of metabolites are detected by this technique: amino acids and acylcarnitines.

Amino acids can identify inborn errors of amino acid metabolism (phenylketonuria, tyrosinemia, maple syrup urine disease, etc.), while the study of the acylcarnitine profile can identify defects of fatty acid oxidation and organic acidemias.

Method

A small punch (one-eighth inch diameter) of the blood collected on filter paper provides the sample needed for MSMS analysis. The sample is then extracted with methanol. After drying, acetonitrile/water are added to the sample that is then injected in the mass spectrometer.

A mass spectrometer measures the ratio of the mass (m) of a chemical and its charge (z). For this reason, all molecules are first ionized usually by electrospray (a process by which molecules are electrically charged). The ions (negatively or positively charged molecules) formed are separated according to their mass to charge ratios. Since most of the ions have one positive charge, their mass to charge ratio corresponds to the mass of the molecules ionized in this process. Two mass spectrometers are used in tandem to separate and analyze mixtures of compounds, such as amino acids or acylcarnitines. After the ions are separated by the first mass spectrometer, they enter the "collision cell" where they are broken down into fragments by collision with a neutral gas. The fragments pass through a second mass spectrometer that separates them according to their mass to charge (m/z) ratio. Each molecule has a characteristic fragmentation pattern and classes of compounds will fragment in a similar way. For example, all acylcarnitines (carnitine conjugated with organic acids or short-, medium-, long-chain fatty acids) generate a fragment of m/z 85. Conversely, all amino acids lose a neutral fragment after fragmentation of m/z 102. The tandem mass spectrometer used for newborn screening is set up to measure only these classes of metabolites (acylcarnitines and amino acids) using the information about their mass and fragmentation pattern. The analysis is very fast (<2 minutes) and suitable for high throughput application, such as newborn screening.

Interpretation of Results

Metabolic disorders are caused by a block in a biochemical pathway, causing the accumulation of disease-specific amino acids or acylcarnitines. With traditional newborn screening methods, samples are flagged when the quantity of a measured metabolite is above a certain value (cutoff). With MSMS, several markers are detected at the same time and the interpretation of the results is based heavily on pattern recognition, while the measurement of the concentration of the different metabolites supports the interpretation (Chace and Kalas 2005). The ability to detect multiple metabolites allows the use of ratios of metabolites to define whether an elevated value is due to a metabolic derangement or to the clinical and nutritional status of the newborn. The increased number of metabolites monitored will result in increased number of results "flagged" as out of range, for which, even though the pattern is not consistent with a metabolic disorder, another screen might be required. Premature infants and infants receiving intravenous hyperalimentation will have a higher rate of out-of-range results, due to the immaturity of their liver and other organs and/or to the components of the intravenous fluids received.

Elevated tyrosine is probably the most commonly found abnormality in premature infants. This is caused by immaturity of the liver, which is the main organ responsible for the catabolism of tyrosine. The abnormality will resolve, in most of these cases, by the time of the routine second screen. Elevated tyrosine is also caused by genetic defects, such as Tyrosinemia type I, II, III, in these cases the routine repeat screen will show an even greater deviation from the normal value.

Intravenous hyperalimentation and/or impaired hepatocellular function are responsible for the increase in several amino acids, such as phenylalanine, methionine, tyrosine, leucine, valine.

Tandem mass spectrometer for newborn screening.
This pattern, in addition to the analysis of specific ratios (phenylalanine/tyrosine; methionine/phenylalanine; leucine/alanine), is easily identified. In these cases a repeat screen might be indicated (after hyperalimentation has been discontinued for at least 2 hours) when the infant is older.

The importance of pattern recognition in the interpretation of MSMS results is better illustrated by the interpretation of acylcarnitine profiles. Carnitine functions as a shuttle to transport long chain fatty acids inside mitochondria where they undergo metabolism. In this process, the fatty acid is shortened producing acetyl-CoA. In addition to this role, carnitine conjugates with organic acids to facilitate their removal. When one step in the metabolism of fatty acids or amino acids/organic acids is impaired, there is an increase in the corresponding acylcarnitine. In MCAD deficiency, a defect in the metabolism of medium chain (6 to 10 carbon atoms) fatty acids, there is an increase in C8-, C6- and C10-carnitine (carnitine conjugated with C8 [octanoic], C6 [hexanoic] and C10 [decanoic] acids). The concentration of C8-carnitine (the marker commonly used for MCAD deficiency in newborn screening programs) is not always higher than the established cut-off. The excessive formation of acylcarnitines in fatty acid oxidation disorders leads to depletion of carnitine resulting in lower concentration of all acylcarnitines and a possible decline of C8-carnitine levels below defined cut-offs.

Interpretation of acylcarnitine profiles, therefore, cannot be based only on cut-off values, because acylcarnitines might not be sufficiently higher than the cut-off value. Further, the same metabolite elevated in the first week of life and decrease rapidly afterwards. Age-appropriate cut-off values are used for the interpretation of the newborn screening results.

Conclusions
Tandem mass spectrometry can presymptomatically identify many metabolic disorders. The interpretation of the results requires personnel familiar with the disorders and the biochemical abnormalities caused by them. 

+Marla Pasquali, PhD, Associate Professor of Phtiology (Clinical) University of Utah School of Medicine, Medical Director, Biochemical Genetics ARUP Laboratories, Supplemental Newborn Screening.

REFERENCES


Key Points of MSMS
- Multiple metabolites can be detected simultaneously with one analysis from one blood spot.
- MSMS allows the identification of several metabolic disorders at once.
- Two main classes of metabolites are detected through MSMS: Amino Acids and Acylcarnitines.
- Premature infants and infants receiving intravenous hyperalimentation (TPN) will have a higher rate of out-of-range results, due to the immaturity of their liver and other organs and/or to the components of the intravenous fluids received.
- Intravenous hyperalimentation and/or impaired hepatocellular function are responsible for the increase in several amino acids, such as phenylalanine, methionine, tyrosine, leucine and valine.
- Another screen is recommended when hyperalimentation has been discontinued for at least 2 hours or when the infant is older.
- In some cases the abnormality reflects a metabolic disorder in the mother.
The information that follows provides a brief description about the genetic basis and inheritance of the metabolic disorders that are detected on expanded newborn screening, as well as implications for other family members.

What causes these conditions?
These conditions are caused by mutations in genes that code for specific enzymes. Enzymes are responsible for breaking down larger substances into smaller units. If an enzyme does not work properly, the substance that it was supposed to breakdown will build up in different organ systems causing various health and medical problems. The enzyme deficiency and related metabolic abnormalities can also lead to problems with normal growth and development. Each condition has a different enzyme that does not function properly. The manifestations and clinical features of each condition will depend upon the specific enzyme that is deficient and the effect of that deficiency on the child's overall health.

Genes act as a blueprint for telling the body how to function properly. We have thousands of genes that code for many different proteins, some of which code for certain enzymes. Mutations in a gene can cause the gene to not function properly, which can result in an enzyme that is deficient or that is not made at all. Everyone has two copies of every gene, one copy inherited from the mother and one from the father. Individuals with these metabolic disorders have a mutation in both copies of the gene that codes for a specific enzyme.

How are these conditions inherited?
All of the expanded newborn screening conditions are inherited in an autosomal recessive manner and can affect both boys and girls equally. Parents of children with one of these metabolic conditions are carriers of the condition. Carriers have one normal copy of the gene and one gene that has been mutated or changed. Carriers do not show clinical signs of the condition because the other gene is working correctly. When both parents are carriers of the same condition, there is a 25% chance in each pregnancy of having a child with that specific metabolic disorder. These children inherit one non-working gene for the condition from each parent (see picture). There is a 50% chance for the child to be a carrier, just like the parents. And, there is a 25% chance for the child to have two working genes.

Genetic counseling is available to families who have children with inborn errors of metabolism and to answer your questions about how they are inherited, choices during future pregnancies, and how to test other family members.

What kind of testing is available?
The specific test that is needed to diagnose these conditions depends upon the metabolic disorder. Some of these tests may include: plasma amino acids, plasma acylcarnitine profile, urine organic acids, and specific enzymatic testing (either on blood or fibroblasts). Genetic testing, which involves identifying the specific mutations within the gene that caused the metabolic disorder, may be available for some of these conditions.
conditions. Genetic testing is often useful for carrier testing of other family members, but is not necessarily required to diagnose the specific metabolic disorder of interest in the affected child. The sensitivity of detecting mutations within the gene depends on the specific condition. More information about available testing options can be obtained by contacting your local metabolic specialist or genetic counselor.

Other family members have an increased risk of being a carrier of the specific condition. The chance that someone is a carrier depends on the relationship of that person to the affected child. Healthy siblings of affected children have a 2/3 chance of being a carrier of the condition. Aunts and uncles of the affected child (siblings of the parents) have a 1/2 chance of being a carrier.

Carrier testing for family members interested in determining their carrier status or for unrelated spouses may be available and can be discussed with the metabolic genetics team. The chance that someone in the general population is a carrier of one of these conditions can range from 1 in 60 to 1 in 200, depending on the specific condition, the incidence in the population, and the ethnic predilection.

What are the reproductive options? Prenatal diagnosis, either via chorionic villi sampling (CVS) or amniocentesis, may be clinically available by enzyme analysis or molecular genetic testing (if both gene changes have been found in the child with the condition). Reproductive testing options can be discussed with a genetic counselor in your area.

*Pilar Lenglet, MS, CGC, University of Utah, Division of Medical Genetics.
Check out the following websites for more information on Acylcarnitine/Amino Acid and newborn screening.

- Utah Department of Health, Newborn Screening Follow Up Program
  http://health.utah.gov/newbornscreening

- National Organization for Rare Disorders
  http://www.rarediseases.org

- Expanded Newborn Screening using Tandem Mass Spectrometry
  http://www.newbornscreening.info/
# Metabolic Disorders Identified by Expanded Newborn Screening

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<th>Abnormal Metabolite</th>
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<th>Recommended Follow Up</th>
<th>Signs/Symptoms</th>
<th>Therapy</th>
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<tr>
<td>Arginine</td>
<td>Arginase deficiency, Liver disease, Hyperalimentation, Prematurity</td>
<td>Plasma ammonia, plasma amino acids, urine orotic acid.</td>
<td>Usually asymptomatic in the neonatal period, mental retardation, spasticity</td>
<td>Low-protein diet, benzoylacetate, phenylbutyrate</td>
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<tr>
<td>Citrulline</td>
<td>Citrullinemia type 1, Citrullinemia type 2, Arginino succinic aciduria, Pyruvate carboxylase deficiency (French form), Lysinuric protein intolerance, Liver disease, Hyperalimentation Prematurity</td>
<td>Plasma ammonia, plasma amino acids, urine orotic acid.</td>
<td>Refusal to feed, lethargy progressing to coma, hyperammonemia, hepatoencephalopathy, dystonia/dysmetria</td>
<td>Low-protein diet, benzoylacetate, phenylbutyrate</td>
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<tr>
<td>Leucine</td>
<td>Maple Syrup Urine Disease, Hyperalimentation Prematurity</td>
<td>Plasma amino acids. Consultation with a metabolic center to arrange immediate diagnostic testing and treatment.</td>
<td>Refusal to feed, lethargy progressing to coma, brain edema</td>
<td>Diet low in branched chain amino acids, thiamine</td>
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<tr>
<td>Methionine</td>
<td>Homocystinuria, Methionine Adenyltransferase deficiency, Glycine N-methyltransferase deficiency, S-adenosylhomocysteine hydrolase deficiency, Tyrosinemia type 1, Liver disease, Hyperalimentation, Prematurity</td>
<td>Plasma amino acids and total plasma homocysteine.</td>
<td>Usually asymptomatic in the neonatal period, mental retardation, thrombotic, colobomata</td>
<td>Diet low in methionine, pyridoxine, folic acid, betaine</td>
</tr>
<tr>
<td>Phenyllalanine</td>
<td>Phenylketonuria, Biotinidinase defects, Dihydroxybiphenyl reductase, Liver disease, Hyperalimentation, Prematurity</td>
<td>Plasma amino acids, urine neopterin profile, blood DHFR activity</td>
<td>Mental retardation, microcephaly</td>
<td>Diet low in phenylalanine</td>
</tr>
<tr>
<td>Proline</td>
<td>Hyperprolinemia type 2, Hyperprolinemia type 1, Lactic acidosis, Liver disease, Hyperalimentation, Prematurity</td>
<td>Plasma amino acids, urine organic acids</td>
<td>Febrile seizures, developmental delays</td>
<td>Pyridoxine (for type 2)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyrosinemia type 1, Tyrosinemia type 2, Tyrosinemia type 3, Liver disease, Hyperalimentation Prematurity</td>
<td>Plasma amino acids and urine organic acids. Consultation with a metabolic center may be necessary.</td>
<td>Liver failure (1), rickets (1), photophobia (2), keratitis (2), delays (2-3)</td>
<td>Diet low in phenylalanine and tyrosine (1-3), NTBC (1)</td>
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<tr>
<td>High C6, C8, C10:1</td>
<td>Medium Chain Acyl CoA Dehydrogenase (MCAD) Deficiency, Diet (special care formulas) Medications</td>
<td>Plasma acylcarnitine profile, urine organic acids, DNA testing.</td>
<td>Hypoglycemia, sudden death</td>
<td>Frequent feedings, low-fat diet, carnitine</td>
</tr>
<tr>
<td>High C14:1, C14</td>
<td>Very Long Chain Acyl CoA Dehydrogenase (VLCD) Deficiency, Intravenous hyperalimentation, Ketonis</td>
<td>Plasma acylcarnitine profile, urine organic acids, enzyme assay in fibroblasts, DNA testing.</td>
<td>Hypoglycemia, cardiomyopathy, sudden death</td>
<td>Frequent feedings, low-fat diet with MCT oil, carnitine</td>
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<td>High C4</td>
<td>Short Chain Acyl CoA Dehydrogenase (SCAD) Deficiency, Isobutyryl CoA dehydrogenase deficiency</td>
<td>Quantitative plasma acylcarnitine profile, urine organic acids, enzyme assay in fibroblasts.</td>
<td>Hypotonia (7)</td>
<td>Frequent feedings, low-fat diet, carnitine</td>
</tr>
<tr>
<td>High C16-OH, C16:1-OH, C18-OH, C18:1-OH</td>
<td>Long Chain 3-OH Acyl CoA Dehydrogenase Deficiency, Seizis, Ketosis</td>
<td>Plasma carnitine, plasma acylcarnitine profile, urine organic acids, 3-OH-fatty acids, DNA testing.</td>
<td>Hypoglycemia, cardiomyopathy, sudden death</td>
<td>Frequent feedings, low-fat diet with MCT oil, low-dose carnitine</td>
</tr>
<tr>
<td>Low free carnitine (C0) and C2</td>
<td>Primary carnitine deficiency, Maternal Primary carnitine deficiency, Prematurity, Infant of vegan, mother, Medications</td>
<td>Plasma carnitine levels, carnitine transport in fibroblasts.</td>
<td>Hypoglycemia, cardiomyopathy, hypotonia, sudden death</td>
<td>Carnitine</td>
</tr>
<tr>
<td>High free carnitine/(C16+C18)</td>
<td>Carnitine Palmitoyl Transferase I Deficiency, Seizis, Carnitine supplementation</td>
<td>Plasma carnitine, plasma acylcarnitine profile, urine organic acids.</td>
<td>Hypoglycemia</td>
<td>Frequent feedings, low-fat diet with MCT oil</td>
</tr>
<tr>
<td>High C16, C16:1, C18, C18:1, low free carnitine</td>
<td>Carnitine Palmitoyl Transferase 2 (CPT2) Deficiency, Carnitine Acylcarnitine Translocase (CACT) Deficiency, Medications</td>
<td>Plasma carnitine, plasma acylcarnitine profile, urine organic acids, DNA testing, enzyme/transporter assay in fibroblasts</td>
<td>Hypoglycemia, cardiac arrest, cardiomyopathy, dysmorphism</td>
<td>Frequent feedings, low-fat diet with MCT oil, carnitine</td>
</tr>
<tr>
<td>High C4, C5, C5Dc, C6, C8, C10, C16</td>
<td>Multiple Acyl CoA Dehydrogenase (MADD) Deficiency, Toxins</td>
<td>Plasma carnitine, plasma acylcarnitine profile, urine organic acids, fibroblast studies</td>
<td>Hypoglycemia, metabolic acidosis, recurrent vomiting, hepatomegaly</td>
<td>Frequent feedings, low-fat diet with MCT oil, carnitine, riboflavin</td>
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<tr>
<td>High C3, C3/C16</td>
<td>Propionic Acidemia, Methylmalonic acidemia, Holoammonase synthase deficiency, Prematurity Diet low in vitamin B12</td>
<td>Plasma ammonia, basic metabolic panel, plasma amino acids, plasma acylcarnitine profile, urine organic acids, serum biotinidase.</td>
<td>Metabolic acidosis, hyperammonemia, coma, hypotonia or hypertonia</td>
<td>Special diet, carnitine, vitamin B12, biotin, other vitamins</td>
</tr>
<tr>
<td>High C5</td>
<td>Isovaleric acidemia (Leucine metabolism), 2-Methylbutyryl-CoA dehydrogenase deficiency (Isolucine metabolism)</td>
<td>Plasma ammonia, basic metabolic panel, plasma amino acids, plasma acylcarnitine profile, urine organic acids and acylcarnitine profile.</td>
<td>Metabolic acidosis, hyperammonemia, coma</td>
<td>Special diet, carnitine, glycine</td>
</tr>
<tr>
<td>High C4</td>
<td>Isovaleryl CoA dehydrogenase deficiency (Valine metabolism), SCAD Deficiency, Medications</td>
<td>Plasma acylcarnitine profile, plasma amino acids, urine organic acids, acylcarnitine and acylcarnitine profile.</td>
<td>Failure to thrive, carnitine deficiency, cardiomyopathy</td>
<td>Carnitine</td>
</tr>
<tr>
<td>High C5:1</td>
<td>2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (Isolucine metabolism), Prematurity</td>
<td>Plasma acylcarnitine profile, plasma amino acids, urine organic acids and acylglycines.</td>
<td>Lactic acidosis, developmental regression, blindness, myoclonic seizures, X-linked</td>
<td>Low-isoleucine diet, carnitine</td>
</tr>
<tr>
<td>High C5Dc</td>
<td>Glutaric acidemia type 1, Diet, Medications</td>
<td>Plasma acylcarnitine profile, urine organic acids and acylcarnitine profile.</td>
<td>Macrocephaly, brain atrophy, hypotonia, dystonia, degeneration of basal ganglia</td>
<td>Special diet, carnitine, vigorous treatment of fever and infections</td>
</tr>
<tr>
<td>High C5-OH</td>
<td>3-Methylcrotonyl CoA carboxylase (MCC) deficiency (Leucine metabolism), Maternal MCC deficiency, Prematurity</td>
<td>Plasma acylcarnitine profile, urine organic acids and acylglycine profile.</td>
<td>Developmental delays, metabolic acidosis, hypoglycemia</td>
<td>Carnitine, Low-protein diet</td>
</tr>
<tr>
<td>High C5-OH, C5:1</td>
<td>3-Ketoisovaleric acidemia (Isolucine metabolism), Ketosis</td>
<td>Plasma ammonia, basic metabolic panel, plasma amino acids, plasma acylcarnitine profile, urine organic acids.</td>
<td>Metabolic acidosis, vomiting, headaches, occasional hyperammonemia and hypoglycemia</td>
<td>Low-protein diet, fasting avoidance, carnitine</td>
</tr>
<tr>
<td>High C6-DC, C5-OH</td>
<td>3-OH 3-CH3 glutaryl CoA Lyase deficiency, Prematurity, Ketosis</td>
<td>Comprehensive metabolic panel, ammonia, plasma amino acids, plasma acylcarnitine profile, urine organic acids.</td>
<td>Hypoglycemia, Mental retardation, epilepsy</td>
<td>Fasting avoidance, carnitine, IV Glucose, vigorous treatment of fever and infections</td>
</tr>
</tbody>
</table>