

Newborn Screening News

A NEWSLETTER OF THE NEWBORN SCREENING PROGRAM AND THE NEWBORN SCREENING LABORATORY

Fall 2005

Tandem Mass Spectrometry (MSMS):

Testing Starts January 2006

FATTY ACID OXIDATION DISORDERS
ORGANIC ACID DISORDERS
AMINO ACID DISORDERS

CUD, MADD, SCAD, MCAD, LCHAD, VLCAD, CARNITINE-ACYLCARNITINE TRANSLOCASE DEFICIENCY, CARNITINE PALMITOYL TRANSFERASE-1 DEFICIENCY, ARGINASE DEFICIENCY, ASA, CIT, HOMOCYSTINURIA, PKU, TYR, BETA-KETOTHIOLASE DEFICIENCY, GA-1, TYPE 1 ISOBUTYRYL COA DEHYDROGENASE DEFICIENCY, IVA, MALONIC ACIDURIA, MSUD, CBL A, CBL B, PA, HMG, 2-METHYL-3-HYDROXYBUTYRYL COA DEHYDROGENASE DEFICIENCY 2-METHYLBUTYRYL COA DEHYDROGENASE DEFICIENCY, MCD

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 acidemias. The abnormal organic acids formed as the result of the

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,

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 (glycine, 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 acidemias, 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 acidemias.

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 Acidemias.

Organic acids are chemicals produced in the intermediate metabolism of amino acids, nucleotides, and fatty acids. Organic acidemias are all autosomal recessive and the affected infant can be the first within a family. Disorders such as propionic acidemia, methylmalonic acidemia, isovaleric

induced vomiting, neurologic dysfunction, and hyperammonemia occur in many disorders of urea cycle intermediates. Metabolic ketoacidosis, often accompanied by hyperammonemia, is a frequent presenting finding in disorders of branched-chain amino acid metabolism (that cause organic acidemias). Occasional disorders produce focal tissue or organ involvement such as liver disease, renal failure, cutaneous abnormalities, or ocular lesions.

Diagnosis of fatty acid oxidation disorders, organic acidemias, or amino acidopathies is confirmed by measuring urine organic acids, plasma carnitine levels (quantitative), 3-OH-fatty acids, plasma acylcarnitine profile, and plasma amino acids. DNA testing and enzyme assays are available for further confirmation of most of these conditions.

Treatment of fatty acid oxidation disorders consists in the avoidance of fasting, low-fat diet sometimes supplemented with specific types of fat such as medium-chain triglycerides that can enter mitochondria independently from carnitine, and carnitine supplementation. In addition, conditions increasing catabolism (such as fever, vomiting, infections) need to be aggressively treated with causative therapy and intravenous glucose when the child is unable to eat.

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 palmitoyl 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. 

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Principles and Results Interpretation [†]By Marzia Pasquali, PhD

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 (cut-off). 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

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.

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

MCAD deficiency (C8-carnitine) is elevated in other diseases (Multiple Acyl-CoA Dehydrogenase Deficiency, MADD) or result from drug therapy (valproic acid) or dietary supplements (MCT oils used in special care formulas for premature infants). In the same way that the amino acid profile aids in the detection of true PKU, the full acylcarnitine profile aids in the detection of disorders of fatty acid oxidation and organic acidemias. Ratios of key metabolites, as well as the absolute values of them, are widely used to ascertain true positive results. In some cases, the abnormality observed in the infant's newborn screening test, reflects a metabolic disorder in the mother. This has been described for two different disorders: 1) 3-

methylcrotonyl-CoA carboxylase deficiency (3 methylcrotonylglycinuria), an inherited disorder of leucine metabolism; 2) primary carnitine deficiency, an inherited disorder of carnitine uptake affecting fatty acid oxidation.

C5OH (3-hydroxyisovaleryl) carnitine is elevated in infants with 3-methylcrotonyl-CoA carboxylase deficiency and remains elevated in subsequent screens. Confirmatory tests in these infants will also be positive. By contrast, infants of mothers with the disease will have normal subsequent screens or confirmatory tests (Koeberl, Millington et al. 2003).

In patients with primary carnitine deficiency the concentration of free carnitine is low and will be even lower in subsequent screens or confirmatory tests. In infants of mothers with primary carnitine deficiency, carnitine levels might normalize over time even without therapy. For these two conditions it is indicated to perform additional tests on the mother as well as on the baby. While amino acids concentrations do not change significantly with age, acylcarnitine concentrations vary significantly. For most acylcarnitines, their concentrations are highest 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 pre-symptomatically identify many metabolic disorders. The interpretation of the results requires personnel familiar with the disorders and the biochemical abnormalities caused by them. 

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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.

Expanded Newborn Screen and Genetics

♦Pilar A. Lenglet, MS, CGC

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.

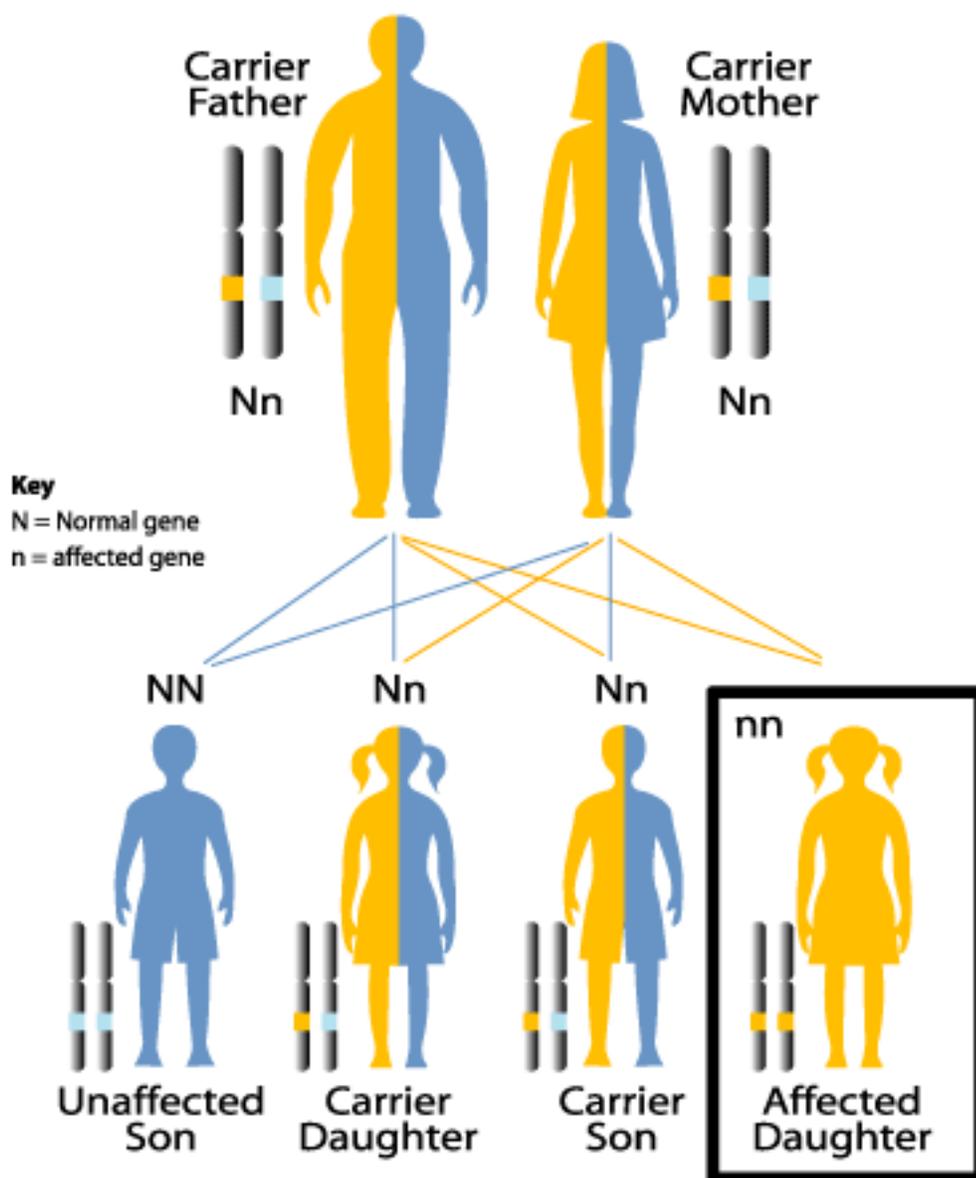
Genetic counseling is available to families who have children with inborn errors of metabolism and to

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

Autosomal Recessive



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.

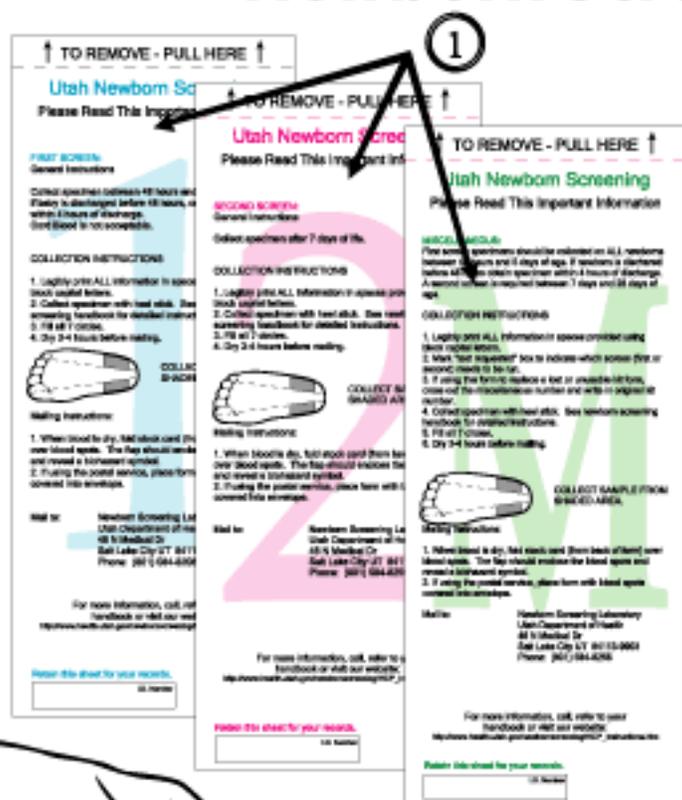
Carrier testing for family members interested in determining their carrier status or for unrelated spouses may be available and can be discussed with the metabolic genetics team. The chance that someone in the general population is a carrier of one of these conditions can range from 1 in 60 to 1 in 200,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

population, and the ethnic predilection.

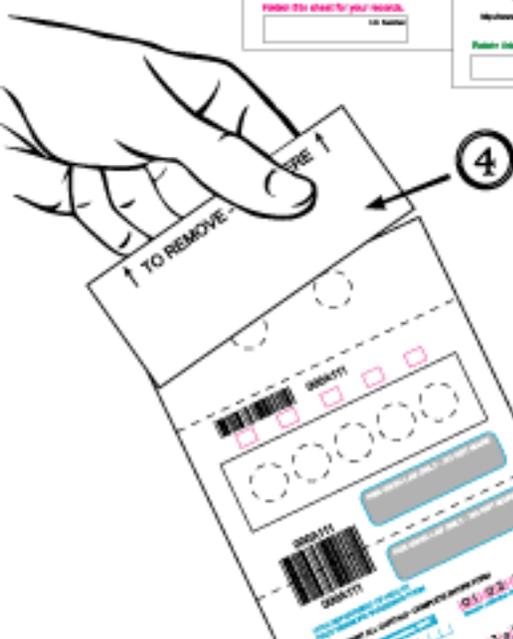
What are the reproductive options? Prenatal diagnosis, either via chorionic villi sampling (CVS) or amniocentesis, may be clinically available by enzyme analysis or molecular genetic testing (if both gene changes have been found in the child with the condition). Reproductive testing options can be discussed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

✦ Pilar Lenglet, MS, CGC, University of Utah, Division of Medical Genetics.

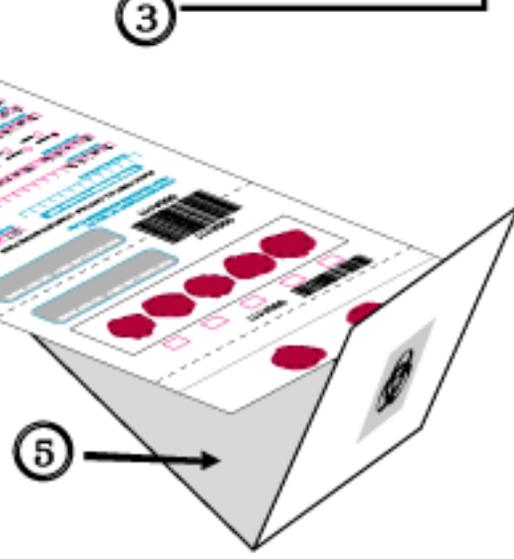
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A Newsletter of the Newborn Screening Program
and the Newborn Screening Laboratory
Utah Department of Health

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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**
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Quick Facts



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Metabolic Disorders Identified by Expanded Newborn Screening

Abnormal Metabolite	Possible Causes	Recommended Follow Up	Signs/Symptoms	Therapy
Arginine	Arginase deficiency, Liver disease, Hyperaminoacidemia, Prematurity	Plasma ammonia, plasma amino acids, urine orotic acid.	Usually asymptomatic in the neonatal period, mental retardation, spasticity	Low-protein diet, benzoate, phenylbutyrate
Citrulline	Citrullinemia type 1, Citrullinemia type 2, Arginino succinic aciduria, Pyruvate carboxylase deficiency (French form), Lysimuric protein intolerance, Liver disease, Hyperaminoacidemia, Prematurity	Plasma ammonia, plasma amino acids, urine orotic acid.	Refusal to feed, lethargy progressing to coma, hyperaminoacidemia, hepatocellular dysfunction	Low-protein diet, benzoate, phenylbutyrate
Leucine	Maple Syrup Urine Disease, Hyperaminoacidemia, Prematurity	Plasma amino acids. Consultation with a metabolic center to arrange immediate diagnostic testing and treatment.	Refusal to feed, lethargy progressing to coma, brain edema	Diet low in branched chain amino acids, thiamine
Methionine	Homocystinuria, Methionine Adenyltransferase deficiency, Glycine N-methyltransferase deficiency, S-adenosylhomocysteine hydrolase deficiency, Tyrosinemia type 1, Liver disease, Hyperaminoacidemia, Prematurity	Plasma amino acids and total plasma homocysteine.	Usually asymptomatic in the neonatal period, mental retardation, thrombosis, cataracts	Diet low in methionine, pyridoxine, folic acid, betaine
Phenylalanine	Phenylketonuria, Bipterin synthesis defects, Dihydrobiopterin reductase, Liver disease, Hyperaminoacidemia, Prematurity	Plasma amino acids, urine neopterin profile, blood DHPR activity	Mental retardation, macrocephaly	Diet low in phenylalanine
Proline	Hyperprolinemia type 2, Hyperprolinemia type 1, Lactic acidosis, Liver disease, Hyperaminoacidemia, Prematurity	Plasma amino acids, urine organic acids	Febrile seizures, developmental delays	Pyridoxine (for type 2)
Tyrosine	Tyrosinemia type 1, Tyrosinemia type 2, Tyrosinemia type 3, Liver disease, Prematurity, Hyperaminoacidemia	Plasma amino acids and urine organic acids. Consultation with a metabolic center may be necessary.	Liver failure (1), rickets (1), photophobia (2), keratosis (2), delays (2,3)	Diet low in phenylalanine and tyrosine (1-3), NTBC (1)
Abnormal Metabolite	Possible Causes	Recommended Follow Up	Signs/Symptoms	Therapy
High C6, C8, C10:1	Fatty Acid Oxidation Defects	Plasma acylcarnitine profile, urine organic acids, DNA testing.	Hypoglycemia, sudden death	Frequent feedings, low-fat diet, carnitine
High C14:1, C14	Very Long Chain Acyl CoA Dehydrogenase (VLCAD) Deficiency, Intrahepatic hyperaminoacidemia, Ketosis	Plasma acylcarnitine profile, urine organic acids, enzyme assay in fibroblasts, DNA testing.	Hypoglycemia, cardiomyopathy, sudden death	Frequent feedings, low-fat diet with MCT oil, carnitine

Abnormal Metabolite	Possible Causes	Recommended Follow Up	Signs/Symptoms	Therapy
High C4	Short Chain Acyl CoA Dehydrogenase (SCAD) Deficiency, Isobutyryl CoA dehydrogenase deficiency	Quantitative plasma acylcarnitine profile, urine organic acids, enzyme assay in fibroblasts.	Hypotonia (?)	Frequent feedings, low-fat diet, carnitine
High C16-OH, C16:1-OH, C18-OH, C18:1-OH	Long Chain 3-OH Acyl CoA, Dehydrogenase Deficiency, Sepsis, Ketosis	Plasma carnitine, plasma acylcarnitine profile, urine organic acids, 3-OH-fatty acids, DNA testing.	Hypoglycemia, cardiomyopathy, sudden death	Frequent feedings, low-fat diet with MCT oil, low-dose carnitine
Low free carnitine (C0) and C2	Primary carnitine deficiency, Maternal Primary carnitine deficiency, Prematurity, Infant of vegan, mother, Medications	Plasma carnitine levels, carnitine transport in fibroblasts.	Hypoglycemia, cardiomyopathy, hypotonia, sudden death	Carnitine
High free carnitine/(C16+C18)	Carnitine Palmitoyl Transferase I Deficiency, Sepsis, Carnitine supplementation	Plasma carnitine, plasma acylcarnitine profile, urine organic acids.	Hypoglycemia	Frequent feedings, low-fat diet with MCT oil
High C16, C16:1, C18, C18:1, low free carnitine	Carnitine Palmitoyl Transferase 2 (CPT2) Deficiency, Carnitine Acylcarnitine Translocase (CACT) Deficiency, Medications	Plasma carnitine, plasma acylcarnitine profile, urine organic acids, DNA testing, enzyme/transporter assay in fibroblasts	Hypoglycemia, cardiac arrest, cardiomyopathy, dysmorphism	Frequent feedings, low-fat diet with MCT oil, carnitine
High C4, C5, C5DC, C6, C8, C10, C16	Multiple Acyl CoA Dehydrogenase (MADD) Deficiency, Toxins	Plasma carnitine, plasma acylcarnitine profile, urine organic acids, fibroblast studies	Hypoglycemia, metabolic acidosis, recurrent vomiting, hepatomegaly	Frequent feedings, low-fat diet with MCT oil, carnitine, riboflavin
Abnormal Metabolite	Possible Causes	Recommended Follow Up	Signs/Symptoms	Therapy
	Organic Acid Disorders			
High C3, C3/C16	Propionic Acidemia, Methylmalonic acidemia, Homocystylase synthase deficiency, Prematurity Diet low in vitamin B12	Plasma ammonia, basic metabolic panel, plasma amino acids, plasma acylcarnitine profile, urine organic acids, serum biotinidase.	Metabolic acidosis, hyperammonemia, coma, hypotonia or hypertonia	Special diet, carnitine, vitamin B12, biotin, other vitamins
High C5	Isovaleric acidemia (Leucine metabolism), 2-Methylbutyryl-CoA dehydrogenase deficiency (Isoleucine metabolism)	Plasma ammonia, basic metabolic panel, plasma amino acids, plasma acylcarnitine profile, urine organic acids and acylglycine profile	Metabolic acidosis, hyperammonemia, coma	Special diet, carnitine, glycine
High C4	Isobutyryl CoA dehydrogenase deficiency (Valine metabolism), SCAD Deficiency, Medications	Plasma acylcarnitine profile, plasma amino acids, urine organic acids, acylglycine and acylcarnitine profile.	Failure to thrive, carnitine deficiency, cardiomyopathy	Carnitine
High C5:1	2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (Isoleucine metabolism), Prematurity	Plasma acylcarnitine profile, plasma amino acids, urine organic acids and acylglycines.	Lactic acidosis, developmental regression, blindness, myoclonic seizures, X-linked	Low-isooleucine diet, carnitine
High C5DC	Glutaric acidemia type 1, Diet, Medications	Plasma acylcarnitine profile, urine organic acids and acylcarnitine profile.	Macrocephaly, brain atrophy, hypotonia, dystonia, degeneration of basal ganglia	Special diet, carnitine, vigorous treatment of fever and infections
High C5-OH	3-Methylcrotonyl CoA carboxylase (MCC) deficiency (Leucine metabolism), Maternal MCC deficiency, Prematurity	Plasma acylcarnitine profile, urine organic acids and acylglycine profile.	Developmental delays, metabolic acidosis, hypoglycemia	Carnitine, Low-protein diet
High C5-OH, C5:1	3-Ketothiolase deficiency (Isoleucine metabolism), Ketosis	Plasma ammonia, basic metabolic panel, plasma amino acids, plasma acylcarnitine profile, urine organic acids.	Metabolic acidosis, vomiting, headaches, occasional hyperammonemia and hypoglycemia	Low-protein diet, fasting avoidance, carnitine
High C6-DC, C5-OH	3-OH-3-C13 glutaryl CoA Lyase deficiency, Prematurity, Ketosis	Comprehensive metabolic panel, ammonia plasma amino acids, plasma acylcarnitine profile, urine organic acids.	Hypoglycemia, Mental retardation, epilepsy	Fasting avoidance, carnitine, IV Glucose, vigorous treatment of fever and infections