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An Overview of Expanded Newborn Screening for Inborn Errors of Metabolism



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An executive summary from the American College of Medical Genetics on newborn screening recommends a core panel of disorders, which meet the criteria for screening. States have used this report as a guide to expand screening programs. The expansion of newborn screening has an impact on primary care physicians who provide the initial contact to families when a child with an abnormal screening result is identified. This article provides an overview of the expanded panel, the screening process, and follow-up treatment guidelines for inborn errors of metabolism included in the core panel. Also included are Internet resources, which provide reliable information for physicians and families.

INTRODUCTION

Newborn screening for metabolic disorders has been in place since the early 1960's when screening began for PKU using the "Guthrie test," developed by Robert Guthrie (1). Congenital hypothyroidism, galactosemia, and sickling hemoglobinopathies were later added to many newborn-screening programs. Although the same blood spot mecha-

nism for collecting samples is used, the development of tandem mass spectrometry (MS/MS) allows us to identify abnormal analytes associated with a variety of disorders of amino acid, organic acid and fat metabolism. Often mistakenly referred to as the "PKU test," newborn screening has advanced such that knowledge of the scope of testing, as well as the recommended confirmatory follow-up is helpful in order for the primary care physician to provide the first line of communication to families who are identified with an abnormal result.

This article provides an overview of expanded newborn screening procedures and follow-up, focus-

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ing on inborn errors of metabolism for which MS/MS screening is available. Galactosemia and biotinidase deficiency, both of which are identified through different methodology but are part of the metabolic screening panel in most states are also reviewed.

There are no mandatory national newborn screening standards in the United States. Each state or territory developed a newborn screening program for infants born under their jurisdiction. In 1999 the American Academy of Pediatrics (AAP) Newborn Screening Task Force indicated the need for uniformity as a result of the disparities in screening services available to infants. In response to this recommendation, the Maternal and Child Health Bureau (MCHB) commissioned the American College of Medical Genetics (ACMG) to outline a process of standardization of outcomes and guidelines for state newborn screening programs, which included the development of a panel of conditions to be included (2). As a result of the work of the newborn screening task force, there are recommendations for a core panel of 29 conditions which meet the criteria for screening.

The criteria are as follows:

- it can be identified at an age at which it may not be clinically detected,
- a test with appropriate sensitivity and specificity is available for it, and
- there are demonstrated benefits of early detection, timely intervention and efficacious treatment of the condition being tested (2).

In addition, there are 25 conditions that are part of the differential diagnosis of abnormal screening results for the core panel of conditions (Table 1). These disorders may be detected in the process of screening for the 29 "recommended" conditions. This report has prompted many states to expand their newborn screening (NBS) panels to comply with those recommendations.

As the primary care provider (PCP), pediatricians and family practice physicians need to be prepared to help families understand the potential implications of an abnormal newborn screen, and assist them in understanding the screening process, diagnostic testing and symptoms. An understanding by the primary care physician of the genetic services available to help the families through the process of diagnosis and treat-

ment can be a reassurance (3). Parents who voice dissatisfaction with the newborn screening process most often cite concerns about their primary care physician's lack of knowledge about their child's metabolic condition (4). Metabolic treatment centers associated with newborn screening programs are staffed by one or more physicians, nutritionists, nurses and genetic counselors trained to manage these rare disorders and are available to provide guidance to the primary care physician. Physicians and local health care facilities may want to familiarize themselves with the screening protocols for their particular state. This information should be available through the health department web site of each state. A national listing of newborn screening programs can be found on the National Newborn Screening and Genetics Research Center (NNSGRC) website. This and other resources available on the internet are listed in Table 2.

SAMPLE COLLECTION

Blood samples collected on filter paper should be obtained 24 hours after birth and prior to discharge from the hospital. The American Academy of Pediatrics recommends a repeat sample be taken within two weeks when the initial sample is collected within the first 24 hours (5). If an extended hospitalization is expected, samples should be collected within the first week of life. If possible, obtain the sample prior to transfusion, since this interferes with some of the test results. Samples collected after transfusion should be noted on the specimen card, and repeated as recommended by state protocol (6).

Instructions for sample collection are printed on filter paper specimen cards. Each circle must be saturated completely, saturating from the topside only. Once filled, cards should be allowed to air dry for several hours prior to mailing. Do not use a heat source to dry samples as heat may destroy enzymes present in the sample. The screening test for galactosemia looks for the presence of enzyme in the blood spot (Table 3). Degradation of the enzyme would result in an abnormal false positive result. Specimens should be mailed or carried by courier to the testing laboratory within 24 hours.

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Table 1
Panel of Disorders Recommended for Screening (2)

MS/MS

ACYLCARNITINES

CORE PANEL

9 OA

Isovaleric Acidemia (VA)
 Glutaric Acidemia I (GA 1)
 Hydroxymethylglutaric Aciduria (HMG)
 Multiple Carboxylase Def. (MCD)
 Methylmalonic Acidemia Mutase Def. (MUT)*
 3-Methylcrotonyl-CoA Carboxylase Def. (3MCC)*
 Methylmalonic Acidemia (Cbl A, B)*
 Propionic Acidemia (PROP)
 β-ketothiolase Def. (BKT)

5 FAO

Medium-chain acyl-Coa Dehydrogenase Def. (MCAD)
 Very long-chain acyl-CoA Dehydrogenase Def. (VLCAD)
 Long-chain L-3-OH acyl-CoA Dehydrogenase Def. (LCHAD)
 Trifunctional Protein Def. (TFP)
 Carnitine Uptake Defect (CUD)

SECONDARY TARGETS

6 OA

Methylmalonic Acidemia (Cbl C, D)*
 Malonic Acidemia (MAL)
 Isobutyryl-CoA-Dehydrogenase Def. (IBG)
 2-Methyl 3-hydroxy Butyric Aciduria (2M3HBA)
 2-Methylbutyryl-CoA-Dehydrogenase Def. (2MBG)
 3-Methylglutaconic Aciduria (3 MGA)

8 FAO

Short-chain acyl-CoA Dehydrogenase Def. (SCAD)
 Glutaric Acidemia Type II (GA2)
 Med/Short-chain L-3-OH acyl-CoA Dehydrogenase Def. (M/SCHAD)
 Med.-chain Ketoacyl-CoA Thiolase Def. (MCKAT)
 Carnitine Palmitoyltransferase II Def. (CPT II)
 Carnitine Acylcarnitine Translocase Def. (CACT)
 Carnitine Palmitoyltransferase I Def. (CPT IA)
 Dienoyl-CoA Reductase Def. (DE RED)

NOTE: Codes are as follows: OA, disorders of organic acid metabolism; FAO, disorders of fatty acid metabolism

*Identifies conditions for which specific discussions of unique issues are found in the main report
<http://www.mchb.hrsa.gov/screening/summary.htm>. Accessed 6/14/06

REPORTING PROCESS

Screening results fall into one of three categories: normal (no elevations of analytes, enzymes present), abnormal (mild elevations), and critical, which indicates a need for swift follow-up. Normal results are usually reported back to the PCP listed on the screening slip, or collection institution by written report. Abnormal results are reported back to the PCP or collection institution for repeat. If subsequent test results are

abnormal, follow-up by the metabolic treatment center (MTC) for definitive diagnosis is recommended.

Critical results are usually reported directly to the MTC for rapid follow-up. The staff at the treatment center contacts the PCP who is asked to contact the family to report the result and perform a physical exam. The metabolic treatment center can give the PCP guidance as to the disorder of concern and subsequent testing to be performed. Publications to guide the PCP through the

AMINO ACIDS

6 AA

Phenylketonuria (PKU)
 Maple Syrup Urine Disease (MSUD)
 Homocystinuria (HCY)*
 Citrullinemia (CIT)
 Argininosuccinic Acidemia (ASA)
 Tyrosinemia Type I (TYR 1)*

3 Hb Pathies

Sickle Cell Anemia (Hb SS)*
 Hb S/β-thalassemia (Hb-SβTh)*
 Hb S/C Disease (Hb S/C)*

6 Others

Congenital Hypothyroidism (CH)
 Biotinidase Def. (BIOT)
 Congenital Adrenal Hyperplasia (CAH)*
 Classical Galactosemia (GALT)
 Hearing screening (HEAR)
 Cystic Fibrosis (CF)

8 AA

Benign Hyperphenylalaninemia (HYPER-PHE)
 Tyrosinemia Type II (TYR II)
 Defects of Biopterin Cofactor Biosynthesis
 (BIOPT (BS))
 Argininemia (ARG)
 Tyrosinemia Type III (TYR III)
 Defects of Biopterin Cofactor Regeneration
 (BIOPT (REG))
 Hypermethioninemia (MET)
 Citrullinemia Type II (CIT II)

1 Hb Pathies

Variant Hb-pathies (Var Hb)*

2 Others

Galactokinase Def. (GALK)
 Galactose Epimerase Def. (GALE)

NOTE: AA, disorders of amino acid metabolism; Hb Pathies, hemoglobinopathies.

*Identifies conditions for which specific discussions of unique issues are found in the main report
<http://www.mchb.hrsa.gov/screening/summary.htm>. Accessed 6/14/06

complexities of inborn errors of metabolism are beyond the scope of this article, but can be found at various web sites (Table 2) and in print (7). In our experience, by the time a family is seen at the metabolic treatment center, they have received information from outside sources such as the internet or other family members. This information can cause distress to parents. Guidance from the local physician can reduce stress by reassuring families that screening is performed to diminish complications

and begin effective treatment prior to symptoms. Internet sources are available to provide patient-friendly education regarding many of the disorders, identified through screening (Table 2).

FOLLOW-UP OF CRITICAL RESULTS

With few exceptions, inborn errors of metabolism are inherited in an autosomal recessive pattern. In this article they will be divided into groups according to their

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Table 2
Online Resources

<i>Sponsoring Agency/URL</i>	<i>Content</i>
National Newborn Screening and Genetics Resource Center http://genes-r-us.uthscsa.edu/	Links to state programs, commercial labs, parent and HCP education materials
Health Resources and Services Administration (HRSA) http://www.newbornscreening.info/	Disorder descriptions for health care professional and parents with links
Save Babies Through Screening Foundation, Inc. http://www.savebabies.org/	Parent oriented site with reviews of disorders, and screening methodologies
National Institutes of Health, HRSA http://www.genetests.org	Reviews of selected genetic disorders, power point presentations, commercial lab information
Fatty Acid Oxidation Support Group http://www.fodsupport.org/index.htm	Parent oriented site for FAODs
HRSA http://mchb.hrsa.gov/programs/genetics/presentations	Information on expanded screening
March of Dimes http://www.marchofdimes.com/printableArticles	Quick reference for HCP on the 29 disorders included in the core panel

Table 3
Galactosemia Screening Process

<i>Results</i>	<i>Beutler (GALT* Enzyme)</i>	<i>Hill</i>	<i>Follow-up Recommended</i>	<i>Treatment</i>	<i>Confirmation</i>
Normal	Enzyme Activity Seen	No elevation of Gal-1-P***	None	No changes	N/A
Abnormal	No Enzyme or Reduced Activity	No elevation (or slight elevation) of Gal-1-P	Possible Galactosemia Variant; evaluate infant and repeat screening test or follow-up at MTC**	Possible elimination of dietary galactose for first year	Quantitative plasma Gal-1-P*** quantitative enzyme activity and mutation analysis
Critical	No Enzyme Activity	Elevated Gal-1-P; may be in Critical Range	Possible Classic Galactosemia, PCP examine infant, MTC perform confirmation testing	Elimination of galactose from diet; switch to soy formula	

*Galactose-1-Phosphate Uridyl Transferase; **Metabolic Treatment Center; ***Galactose-1-Phosphate

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critical substrates. Children with metabolic disorders need the on-going care of their PCP as well as the MTC. A cooperative effort between them provides the best care for the patient.

DISORDERS OF AMINO ACID OR PROTEIN METABOLISM

Disorders of amino acid metabolism are caused by enzyme deficiencies resulting in the accumulation of metabolites to levels which cause organ damage. The brain, liver and kidneys are the most frequently affected organs (8). Symptoms may be acutely present or become apparent weeks or months after birth as a result of the cumulative effect of damage. Table 4 lists the aminoacidopathies included in the recommended NBS panel, along with their presentation and treatment. Treatment consists of dietary restriction of offending amino acid(s) along with disease-specific medical formula that provides adequate protein for growth and development. With strict dietary adherence, outcome is generally good.

Phenylketonuria

Phenylketonuria (PKU) is caused by a deficiency of the enzyme phenylalanine hydroxylase that converts

phenylalanine to tyrosine, resulting in an elevation of plasma phenylalanine. Children with PKU who successfully maintain plasma phenylalanine levels within treatment range are able to develop normally and perform in school as well as their non-PKU siblings. Parents should be reassured that treatment is effective, manageable, and that support systems are in place to assist them in caring for their child. When identified and treated within the first weeks of life, outcome is good. Treatment includes dietary restriction of protein, and a metabolic formula specific to PKU that does not contain phenylalanine. This formula, in combination with the restriction of natural protein, provides the child with the necessary nutrition to support normal growth and development. Damaging effects of elevated plasma phenylalanine do not become apparent unless levels continue to be elevated for months after birth. Newborn screening has been an effective method of preventing the neurological complications once associated with PKU (9).

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD) is a disorder of branched chain amino acid metabolism caused by a

Table 4
Aminoacidopathies Included in the Recommended NBS Panel (8)

<i>Disorder</i>	<i>Clinical Symptoms</i>	<i>Onset</i>	<i>Treatment</i>
PKU*	Normal physical exam at birth, Untreated: neurological damage through missed milestones, mental retardation	Gradual	Dietary restriction of phenylalanine; supplementation with tyrosine as needed.
MSUD*	Encephalopathy, lethargy, feeding problems Somnolence, cerebral edema, coma	Neonatal 3–5 days	Dietary restriction of branched chain amino acids, avoid isoleucine and valine deficiency, trial of thiamine to increase enzyme activity
HCY*	Normal newborn physical exam Untreated: lens dislocation, progressive myopia, osteoporosis, Marfan-like appearance; risk of stroke	Gradual	Dietary restriction of methionine; Trial of pyridoxine at therapeutic doses; betaine
TYR-I*	Severe liver failure, vomiting, septicemia, hypoglycemia, jaundice, rickets	Early infancy	Dietary phenylalanine + tyrosine restriction; treatment with NTBC

*PKU - Phenylketonuria; MSUD - Maple Syrup Urine Disease; HCY - Homocystinuria; TYR-1 - Tyrosinemia Type I.

Table 5
Organic Acidemias Included in the Recommended NBS Panel (8)

<i>Disorder</i>	<i>Precursor Amino Acid(s)/Substrates</i>
Propionic Acidemia (PA)	Methionine, Isoleucine, Threonine, Valine
Isovaleric Acidemia (IVA)	Leucine
3 Methylcrotonyl CoA Carboxylase Deficiency (3-MCC)	Leucine
Cobalamin defects (Cbl A,B)	Methionine, Isoleucine, Threonine, Valine
Glutaric Aciduria Type I (GA-I)	Lysine, Tryptophan
HMG-Co-A Lyase Deficiency	Leucine; fats

deficiency of branched chain keto-acid dehydrogenase enzymes, resulting in elevated plasma leucine (Leu), isoleucine (Ile) and valine (Val). Children with MSUD are at risk for metabolic crisis within the first few days of life. There is a wide range of clinical presentation. In the severe “classic” form, symptoms will occur within three to five days (Table 4). Treatment consists of a protein-restricted diet and medical formula specific to MSUD that does not contain Leu, Ile, and Val. The restriction of natural protein along with the medical formula provides the essential nutrients to support normal growth and development. Monitoring plasma branched chain amino acids and avoidance of Ile and Val deficiencies is necessary to prevent metabolic crisis (10).

Organic Acidemias

Organic acidemias (Table 5) are a group of inherited metabolic disorders that lead to an accumulation of organic acids that cause disturbance in the acid-base balance and alterations in pathways of intermediary metabolism (8). This group of disorders is associated

with severe, recurrent episodes of clinical and biochemical decompensation. Milder variants are associated with children that do not present during the neonatal period. However, when crisis occurs, it can be equally as lethal as the neonatal form (8). Early detection of these disorders can lead to better outcome through restriction of specific amino acids, therapeutic use of vitamins involved in the metabolic pathway when indicated, aggressive treatment during illness, and avoidance of catabolism. Outcome is dependent upon the frequency and severity of crisis, which often occur during acute illness.

Urea Cycle Disorders

Urea cycle disorders are inherited disorders of nitrogen metabolism involving any one of six enzymes in the nitrogen pathway. Of this group of disorders, only citrullinemia and argininosuccinic acidemia are included in the recommended NBS panel (Table 6). All of the urea cycle disorders are inherited as autosomal recessive disorders with the exception of ornithine transcar-

Table 6
Urea Cycle Disorders included in Recommended NBS Panel

<i>Urea Cycle Disorder</i>	<i>Enzyme</i>	<i>Clinical Presentation</i>
Argininosuccinic Aciduria (ASA)	Argininosuccinate lyase	Lethargy, poor feeding, hyperventilation, seizures, coma, temperature instability, loss of reflexes
Citrullinemia (CIT)	Argininosuccinate synthase	Usually a milder neonatal course; symptoms typically manifest after neonatal period

Table 7
Fatty Acid Oxidation Disorders (in Core Panel)

- Medium Chain acyl CoA Dehydrogenase Deficiency (MCADD)
- Long Chain Hydroxylacyl CoA Dehydrogenase Deficiency (LCHADD)
- Very Long Chain acyl Co-A Dehydrogenase Deficiency (VLCADD)
- Carnitine Uptake Defects (CUD)
- Trifunctional Protein Deficiency (TFP)

bamylase (OTC) deficiency, which is an X-linked trait often lethal in males (11). Symptoms progress rapidly as ammonia accumulates, particularly during catabolic states. Diagnosis is made by quantitative plasma and urine amino acids, measurement of orotic acid in urine, enzyme assays and mutation (DNA) analysis (11). This group of disorders can present at all ages. Neonatal presentation is generally associated with more severe disorders. Treatment for the severe disorders is aimed at protecting neurologic integrity until liver transplant, which can happen as early as during the first year of life.

DISORDERS OF CARBOHYDRATE METABOLISM

Galactosemia is a disorder of galactose metabolism. The classic form results from a severe deficiency of the enzyme galactose-1-phosphate uridyl transferase (GALT). Variants of GALT deficiency are usually milder in presentation and may only require restriction of galactose during the first year of life, when lactose is the major carbohydrate source in the diet. Galactokinase and UDP-galactose-4-epimerase enzyme deficiencies are less common forms of galactosemia. Follow-up testing can determine which enzyme is affected to determine the best treatment course. Treatment is restriction of lactose and galactose from the diet, which is more complicated than elimination of milk and dairy products. Lactose is used in medications as a bulking agent, and galactose is found in many fruits and vegetables. Metabolic treatment centers have

resources for parents or care providers to use in determining foods that are to be avoided.

Accumulation of galactose-1-phosphate is used as a guide to determine the need for dietary restriction. Screening involves both enzyme and metabolite detection (Table 3). The “classic” form of GALT deficiency requires dietary restriction of galactose for life. Infants not identified early may present with symptoms of poor feeding, vomiting and diarrhea, lethargy, disturbance in liver function, jaundice, and bilateral cataracts (8). Approximately 20% of infants with untreated galactosemia will present with gram-negative infections (12). The PCP should evaluate all patients with an abnormal galactosemia newborn screen in order to assure that the infant is healthy and feeding well. Changing feeds from cow’s milk formula to a soy-based formula is recommended pending results of follow-up testing. If mother is breastfeeding, and screening results suggest that the infant has a variant form of galactosemia rather than the “classic” form, the advantages of continued breast feeding need to be weighed against risk of harm to the infant. The metabolic treatment center may be able to help with the decision to continue breast-feeding by interpreting the screening test and facilitating rapid diagnosis.

DISORDERS OF FAT METABOLISM

Fatty acid oxidation disorders (FAOD) are inborn errors of metabolism that involve enzymes along the fatty acid oxidation pathway (Table 7). These errors affect the use of dietary and endogenously stored fat, and present with wide variability. Patients are healthy until a catabolic or fasting state is experienced, at which time they may present with Reye syndrome-like symptoms such as lethargy, nausea and vomiting, progressing to metabolic crisis (8). Five FAODs are included in the recommended NBS panel, with 8 additional FAODs in the secondary list. Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common of the FAOD’s. Avoidance of fasting is the first line of defense against symptoms. Infants with MCAD will need one or two feedings during the night, and on-demand feeds during the day. Older children with MCAD can usually sleep through the night with-

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out developing hypoglycemia, but may need intravenous glucose during times of catabolic stress such as vomiting, fever or diarrhea. Enteral support can be provided in the form of uncooked cornstarch, a slow-release glucose source, prior to sleep. Treatment goal is to maintain normal glycemia and prevent the need for fat as an energy source. Ongoing treatment may include supplementation with carnitine and a low fat diet (20–30% calories from fat), with avoidance of fasting being the most beneficial. Patients with these disorders will often present with nonketotic hypoglycemia due to their inability to use fat as an energy source (8). The short-chain acyl CoA dehydrogenase (SCAD) deficiency is the exception. Neonatal onset has a variable phenotype that includes metabolic acidosis, failure to thrive, developmental delay, and seizures as well as myopathy (13).

BIOTINIDASE DEFICIENCY (MULTIPLE CARBOXYLASE DEFICIENCY OR MCD)

Dr. Barry Wolf demonstrated that the biochemical defect in patients with late onset multiple carboxylase deficiency was caused by deficiency of biotinidase (14). In severe biotinidase deficiency, early symptoms include seizures, hypotonia, alopecia, skin rash, ataxia, hearing impairment, and conjunctivitis (14). As with all of the metabolic disorders, there is wide variability. Patients who are identified with partial deficiency may show no clinical signs. This disorder is sometimes classified as one of the organic acid disorders because of the accumulated metabolites that lead to metabolic crisis if untreated. Screening is not performed using MS/MS technology, but rather a colorimetric enzyme assay of the blood spot sample. Follow-up testing measures serum biotinidase activity. Patients with partial deficiencies have enzyme activity in the 10–30% range, with more severe forms falling below 10% (10). Treatment is daily supplementation with biotin in free form at a dose of 5 to 20 mg/day, which continues for life (14).

CONCLUSION

It is important that the primary care physician maintain an active role in the care of a child with an inborn error of metabolism, providing well and sick child care. Their

expertise in assessing the patient's clinical status and assistance with home monitoring assists the metabolic treatment center in providing optimum care. The metabolic treatment center is a resource both for the family and the primary care physician. As states expand newborn screening, metabolic centers will need to rely on the physician in the community to provide emergency care for the sick child, as well as serve as a patient advocate. A cooperative effort will benefit all involved, and facilitate the best outcome for the patient. ■

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