

Update on Newborn Screening

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Since phenylketonuria was first screened for in the 1960s, newborn screening has expanded to include more than 30 conditions. This commentary provides an update on newborn screening, including the follow-up of abnormal findings, the limitations of such screening, and the ethical questions that screening raises.

Newborn screening began in the 1960s as a way of detecting disorders that did not present with identifiable clinical features but that could be successfully treated if therapy was initiated early. Screening involves obtaining a sample of blood during the first 24 to 48 hours of life and looking for markers indicative of various disorders. A requirement of such screening is that each disorder being screened for must have a sensitive and reliable marker that can be detected by a simple, inexpensive test.

The prototype for newborn screening, which was also the first disorder to be screened for, is phenylketonuria (PKU), an inborn error of metabolism of the amino acid phenylalanine. Without treatment, PKU results in severe intellectual disability; however, if a phenylalanine-restricted, low-protein diet is initiated in the first few weeks of life, then intelligence remains normal or near-normal. PKU was initially screened for using a bacterial inhibition assay developed by Robert Guthrie [1], which relies on placing a blood sample on a plate with bacteria that cannot grow in the presence of high levels of phenylalanine. If there is too much phenylalanine in the sample, growth of the bacteria is inhibited, and the sample will appear to be surrounded by a "halo"—an area where there are no bacteria. The diameter of the zone with no bacterial growth is directly dependent on the amount of phenylalanine. If control amounts of phenylalanine are used in the same assay, then the amount of phenylalanine in the tested samples can be quantified. This method was soon applied to maple syrup urine disease (indicated by the detection of elevated leucine levels) and to homocystinuria (indicated by the detection of elevated methionine levels). In the 1960s, 37 states had laws supporting newborn screening for PKU.

In the 1990s, electrospray tandem mass spectrometry (MS/MS) methods were introduced into state newborn screening programs [2]. This methodology allows multiple analytes to be detected in a single blood spot sample. In addition to measuring levels of phenylalanine, leucine, and methionine, MS/MS can measure levels of other amino

acids to identify certain urea cycle defects: citrullinemia, arginosuccinic aciduria, hyperargininemia, and the 3 types of tyrosinemia. An acylcarnitine profile performed by MS/MS also allows the detection of many organic acidemias, fatty acid oxidation defects, and other carnitine uptake and transporter defects.

All states now have laws mandating newborn screening. Each state independently determines which screening tests are done and what follow-up is provided [3]. In addition, states develop their own policies regarding sample collection, laboratory procedures, result reporting, and follow-up programs and education. Regarding consent for testing, most states have mandatory newborn screening with defined opt-out policies for parents [4].

Newborn screening has been extremely successful in improving the outcomes and decreasing the burden of PKU. With the expansion of newborn screening, however, states now vary significantly in terms of which disorders are screened for. In 1995 states mandated screening for an average of 5 disorders (range, 0–8); in 2005 this panel included anywhere from 4 to 45 disorders, depending on the state [5]. Because of this extreme variability, the Maternal and Child Health Bureau in the Health Resources and Services Administration of the US Department of Health & Human Services requested in 1999 that the American Academy of Pediatrics develop a national task force on newborn screening [6]. Subsequently, the Maternal and Child Health Bureau tasked the American College of Medical Genetics and Genomics (ACMG) with developing a process for standardizing state newborn screening programs, including deciding which conditions to include in the screening panel and providing guidelines for collecting and evaluating screening results [7]. The expert panel identified 29 core conditions and 25 secondary conditions that were to be included in a Recommended Uniform Screening Panel (RUSP). The Secretary of Health and Human Services endorsed the RUSP in 2010. The Secretary's Advisory Committee on Heritable Disorders in Newborns and Children was also formed to pro-

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vide an evidence-based decision-making process for evaluating conditions that are nominated for inclusion in the RUSP [8]. In 2010 severe combined immunodeficiency and critical congenital cyanotic heart disease were recommended by the committee for inclusion in the RUSP and were approved by the Secretary of Health and Human Services, bringing the total number of conditions screened for to 31 (Table 1) [7, 9].

A majority of the disorders screened for require follow-up

with confirmatory blood (biochemical) and/or genetic (molecular) testing by physicians who are experts in those disorders. In some states (including North Carolina), screening for cystic fibrosis actually involves genetic diagnostic testing as part of the screening process. In North Carolina, cystic fibrosis screening is a 2-tiered process. The first tier involves measurement of immunoreactive trypsinogen (IRT) levels. The 5% of samples with the highest IRT levels are then

TABLE 1.
Newborn Screening Panel: Core and Secondary Targets

Core panel				
Tandem mass spectrometry				
Acylcarnitines		Amino acids		
Disorders of organic acid metabolism	Disorders of fatty acid oxidation metabolism	Disorders of amino acid metabolism	Hemoglobinopathies	Other disorders
Isovaleric acidemia	Medium-chain acyl-CoA dehydrogenase deficiency	Phenylketonuria	Sickle-cell anemia	Congenital hypothyroidism
Glutaric acidemia	Very-long-chain acyl-CoA dehydrogenase deficiency	Maple syrup urine disease	Sickle-cell beta thalassemia	Biotinidase deficiency
3-hydroxy 3-methylglutaric aciduria	Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency	Homocystinuria	Hemoglobin SC disease (a variant of sickle-cell anemia)	Congenital adrenal hyperplasia
Multiple carboxylase deficiency	Trifunctional protein deficiency	Citrullinemia		Classic galactosemia
Methylmalonic acidemia caused by methylmalonyl-CoA mutase deficiency	Carnitine uptake defect	Argininosuccinic acidemia		Hearing loss
3-methylcrotonyl-CoA carboxylase deficiency		Tyrosinemia type I		Cystic fibrosis
Methylmalonic acidemia caused by cobalamin disorder A or B				Severe combined immunodeficiency
Propionic acidemia				Critical cyanotic congenital heart disease
Beta-ketothiolase deficiency				
Secondary targets				
Methylmalonic acidemia caused by cobalamin disorder C or D	Short-chain acyl-CoA dehydrogenase deficiency	Benign hyperphenylalanemia	Other variant hemoglobinopathies	Galactokinase deficiency
Malonic acidemia	Glutaric acidemia type II	Tyrosinemia type II		Galactose epimerase deficiency
Isobutyryl-CoA dehydrogenase deficiency	Medium/short-chain 3-hydroxy acyl-CoA dehydrogenase deficiency	Defects of biopterin cofactor biosynthesis		
2-methyl 3-hydroxy butyric aciduria	Medium-chain ketoacyl-CoA thiolase deficiency	Argininemia		
2-methylbutyryl-CoA dehydrogenase deficiency	Carnitine palmitoyltransferase II deficiency	Tyrosinemia type III		
3-methylglutaconic aciduria	Carnitine/acylcarnitine translocase deficiency	Defects of biopterin cofactor regeneration		
	Carnitine palmitoyltransferase IA deficiency	Hypermethioninemia		
	Dienoyl-CoA reductase deficiency	Citrullinemia type II		

Note. CoA, coenzyme A.
Source: Adapted from Watson et al [7].

subjected to a genetic testing panel that looks for more than 40 common cystic fibrosis mutations. An abnormal cystic fibrosis screening result is one in which 1 or 2 mutations are identified or the IRT value is greater than 175 ng/mL. If an abnormal result is found, a recommendation for confirmatory sweat testing is conveyed to the primary care provider.

Genetic testing is expected to become more commonplace in newborn screening in the future. This will require broader understanding on the part of providers, who may have to engage in potentially complicated and confusing discussions with parents about the newborn screening process and the test results. Research indicates that parents prefer that primary care physicians provide open, honest, informed communication about abnormal newborn screening results. Providers who avoid jargon, recognize parental distress, and encourage questions are rated higher. Knowing when to refer the newborn to specialists is also important [10].

Typically, laboratories relay newborn screening results to the primary physician within 2 weeks of receiving the sample. In North Carolina, results can also be accessed online through the newborn screening section of the North Carolina State Laboratory of Public Health (<http://slph.state.nc.us/newborn/Reporting.asp>).

The ACTion (ACT) sheets from the ACMG (<http://www.ncbi.nlm.nih.gov/books/NBK55827/>) are an excellent resource on newborn screening and abnormal screening results. Developed by an expert group with members from various specialties that deal with conditions involved in newborn screening, these sheets have been approved by the board of directors of the ACMG. For each marker, 2 items are provided: an ACT sheet, which describes the short-term actions a health professional should take in communicating with the family and initiating follow-up care of an infant who has screened positive for 1 or more conditions; and an algorithm, which presents an overview of the basic steps involved in determining the final diagnosis for the infant.

Although newborn screening is very effective, it does have limitations. Not all inborn errors of metabolism are included in the newborn metabolic screen, for a variety of reasons. In some instances, there is not an effective method of testing for a disorder using a blood spot sample; in other instances, the level of the metabolite does not differ between affected individuals and unaffected individuals. Another limitation is that screening may produce false-positive results (the screen is positive, but the individual does not have the disease) or false-negative results (the screen is negative, but the individual does have the disease). Because the goal of screening is to minimize the number of cases that are missed, false-positive results are very common; these results may lead to increased parental stress and may impair parent-infant bonding [11]. False-negative results are of even greater concern, because they mean that the screen failed to detect a condition for which early identification and treatment are crucial. Also, some diseases (including maple syrup urine disease, organic acidemias, and urea cycle disorders)

may present clinically in the first couple of days of life, before the newborn screening results are available.

Some of the disorders currently screened for may not have clinical consequences. An elevated level of C5-OH acylcarnitine, which is the marker for 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency, can be detected in the blood spot of infants born to parents who may be carriers (or may have the disorder) but who have never had symptoms. While 3-MCC deficiency may be a common benign condition, identifying a baby as having 3-MCC deficiency may lead to unnecessary parental anxiety and inappropriate "labeling" of the baby.

In a 1968 report to the World Health Organization, Wilson and Jungner outlined 10 criteria that should be met prior to implementing screening for certain disorders [12]. Building on these criteria, the current Advisory Committee on Heritable Disorders in Newborns and Children has developed a process for determining which conditions should be added to the RUSP [8]. Specifically, a scoring system is used that assesses the magnitude and certainty of net benefit of screening (determined by reviewing the evidence in the literature and consulting experts). The committee also evaluates the feasibility of screening and the readiness of state newborn screening programs to adopt screening for the disorder.

Some disorders have been proposed to the Advisory Committee for which no good treatments are available, or for which only experimental treatments are available. Some of these disorders (eg, Krabbe disease, Duchenne muscular dystrophy, and fragile X syndrome) are currently included in pilot screening projects in some states. There is debate over the ethics of screening when the disorder has no proven treatment or cure (Krabbe disease), when disease onset is outside the newborn period (Duchenne muscular dystrophy), or when there are implications for additional family members (fragile X syndrome) [9].

There are additional concerns regarding newborn screening for cystic fibrosis. Unlike most screening tests, which require follow-up testing for diagnosis, the technology used for newborn screening of cystic fibrosis is a diagnostic test. Also, newborn screening for cystic fibrosis identifies not only affected individuals but may also identify carriers, who do not have the disorder, and there is the potential for confusion when relaying these results to parents [13].

Finally, there is debate over what to do with blood spot cards after screening has been completed. The federal regulatory standards of the Clinical Laboratory Improvement Amendments of 1988 require that results and cards be preserved for 2 years to allow for test verification and for quality improvement. How long the cards are stored beyond this period varies from state to state. A related issue is that only about half of states have laws addressing the use of residual sample on blood spot cards. Some states allow the blood to be used for public health and basic scientific research studies without requiring additional parental consent. This

practice is controversial, and legal disputes have occurred in several states [14].

As we celebrate the 50th anniversary of newborn screening, questions remain about how to maximize benefit and minimize harm [15]. While specialists will be primarily responsible for addressing these questions, primary care physicians also need to understand newborn screening in order to explain it to parents. As more disorders are added to the RUSP, physicians are more likely to be contacted with an abnormal screening result. Knowing how to approach these results and how to discuss them with parents will be crucial. Also, as genetic testing becomes more commonplace, newborn screening is increasingly likely to include use of diagnostic tests, so parents will need to be educated about the differences between screening tests and diagnostic tests. **NCMJ**

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