Newborn Screening

History, Current Status, and Future Directions

Ayman W. El-Hattab, MD[a], Mohammed Almannai, MD[b], V. Reid Sutton, MD[b,*]

KEYWORDS

• Inborn errors of metabolism • Newborn screening • Tandem mass spectroscopy

KEY POINTS

- Newborn screening aims to achieve presymptomatic diagnosis of treatable disorders to allow for early initiation of medical care to prevent or reduce significant morbidity and mortality related to the screened disorders.
- Many of the conditions tested in the newborn screening are inborn errors of metabolism; however, a wide variety of other nonmetabolic disorders may be included.
- In the United States, the Advisory Committee on Heritable Disorders in Newborn and Children (ACHDNC) provides recommendations regarding conditions to be included in newborn screening program panels; however, the final decision of which disorders to be added to the newborn screening is typically made by each individual state.
- Newborn screening tests are not designed to be diagnostic. Therefore, further diagnostic tests are needed to confirm or exclude the suspected diagnosis.
- Further advancement in technology is expected to allow continuous expansion of newborn screening with reduction in cost, shorter turnaround time, and more accurate results.

INTRODUCTION

Newborn screening aims to achieve early presymptomatic diagnosis of treatable disorders for which timely intervention is critical to improve the outcome. Many of the conditions included in the newborn screening panels are inborn errors of metabolism; however, screening for endocrine, hematologic, immunologic, and cardiovascular diseases, and hearing loss is also included in many panels. Newborn screening includes point-of-care tests (eg, hearing test) and blood analysis of samples collected on filter

Declaration of Conflict of Interests: The authors declare that there is no conflict of interest.

[a] Division of Clinical Genetics and Metabolic Disorders, Pediatrics Department, Tawam Hospital, Tawam Roundabout, Al-Ain 15258, United Arab Emirates; [b] Department of Molecular and Human Genetics, Baylor College of Medicine, Texas Children's Hospital, One Baylor Plaza, Houston, TX 77030, USA

* Corresponding author.

E-mail address: vrsutton@texaschildrens.org

Pediatr Clin N Am 65 (2018) 389–405

https://doi.org/10.1016/j.pcl.2017.11.013

pediatric.theclinics.com

paper spots between 24 and 48 hours of age. Tests in newborn screening are not designed to be diagnostic. Therefore, abnormal newborn screen results should prompt the initiation of further diagnostic testing, neonate evaluation, and consideration of treatment initiation while waiting for the diagnostic test results.[1–3] This article focuses on newborn screening for inborn errors of metabolism. The goals and history of newborn screening are discussed. Then how disorders are selected for inclusion in newborn screening and how to optimize its results are explained. Logistics, factors affecting newborn screening results, and confirmation process are then presented. Finally, future directions of newborn screening are discussed.

GOALS OF NEWBORN SCREENING

Newborn screening aims to achieve presymptomatic and rapid diagnosis of treatable disorders for which timely intervention is critical to improve the outcome. These conditions are typically not evident at birth and if not diagnosed and treated could result in disability or death. Therefore, the goal of newborn screening is the prevention or reduction of significant morbidity and mortality related to various disorders. Newborn screening programs have enabled early diagnosis and initiation of medical care for the screened diseases, which has modified the outcome for many disorders that were previously associated with high morbidity (eg, inborn errors of metabolism, cystic fibrosis, and primary immunodeficiencies) or with significant neurodevelopmental disabilities (eg, phenylketonuria and congenital hypothyroidism). Improving the outcome for affected children is productive for society and the individual child.[2–4]

Because early diagnosis by newborn screening facilitates early intervention, the outcome of newborn screening programs has been favorable. Several studies of long-term follow-up of individuals ascertained by newborn screening indicated significant improvement in morbidity and mortality for all diseases that have been studied including fatty acid oxidations defects, urea cycle disorders, severe combined immunodeficiency, cystic fibrosis, and sickle cell disease.[5–9]

HISTORY OF NEWBORN SCREENING

The establishment of newborn screening was based on early work in the management of phenylketonuria. The importance of early diagnosis for phenylketonuria emerged when it was observed that individuals with phenylketonuria had improvement in their clinical status when given formulas modified to restrict phenylalanine intake, and such restriction can typically prevent intellectual disability associated with phenylketonuria if started early in life.[10,11] In 1963 Guthrie and Susi[12] reported a simple method for detecting phenylketonuria in large populations of newborns. Not different from today's sampling method, the blood for this test was collected from newborns on filter paper. The analysis method, which is known as bacterial inhibition assay, depended on placing a small punch from the filter paper on an ager plate containing a heavy inoculum of *Bacillus subtilis* bacteria and β_2-thienylalanine, which is an inhibitor of bacterial growth that is counteracted by any significant excess of phenylalanine in the blood sample. Elevated phenylalanine in phenylketonuria reverses the effect of the inhibitor, and the extent of bacteria growth surrounding the filter paper disk is correlated with phenylalanine level in the blood spot.[12] In the same year, Massachusetts began universal mandatory screening for phenylketonuria, and rapidly, other states started establishing newborn screening programs.

Screening tests for other inborn errors of metabolism were subsequently developed. The bacterial inhibition assay was used to detect other inborn errors of metabolism, such as galactosemia, maple syrup urine disease, and homocystinuria,

although they were not as widely adopted as screening for phenylketonuria. Enzyme assays for newborn screening blood spots were then developed for galactosemia in 1968 and biotinidase deficiency in 1984.[13,14] In 1980s fluorimetric assays were developed and replaced bacterial inhibition assays for analyte analysis. In 1990 tandem mass spectroscopy, which had been used clinically to measure urine acylcarnitines, was demonstrated to be amenable to the detection of analytes in newborn screening blood spots.[15] This methodology is highly automated; allows the detection of large number of analytes simultaneously in a single assay; and has high speed of sample preparation, assay, and analysis. Therefore, the adoption of this methodology has revolutionized newborn screening by allowing rapid expansion of the number of diseases included in newborn screening with reduction of cost and turnaround time for testing.

In the United States, the Newborn Screening Saves Lives Act of 2007 was passed by the Congress and signed into law in 2008. Subsequently, seven Regional Genetics and Newborn Screening Service Collaboratives and a National Coordinating Center for the Collaboratives were established to facilitate improvements in education, training, screening technology, and follow-up strategies. This law also established programs for newborn screening improvement, quality assurance, and activities coordination.[16]

SELECTION OF DISORDERS INCLUDED IN NEWBORN SCREENING

Wilson and Jungner[17] published 10 principles that could be used for inclusion of a condition in newborn screening in 1986 (**Box 1**). However, over time a broad and disparate profile of screening targets emerged because each state determines what disorders should be included for screening.

In 2006, the Maternal and Child Health Bureau of the Health Resources and Services Administration and the American College of Medical Genetics and Genomics published practice guidelines for newborn screening to standardize a panel that would be used nationwide. In these guidelines three minimal criteria were used to include a disease as a primary target in newborn screening (**Box 2**), and disorders were evaluated based on their clinical characteristics (eg, incidence, burden of disease if not

Box 1
Wilson and Jungner principles for including a disease in newborn screening

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized diseases.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a "once and for all" project.

Box 2
The American College of Medical Genetics and Genomics minimal criteria for a condition to be included as a primary target in newborn screening

1. It can be identified at a time (24–48 hours after birth) at which it would not ordinarily be detected clinically
2. A test with appropriate sensitivity and specificity is available for it
3. There are demonstrated benefits of early detection, timely intervention, and efficacious treatment of the condition.

treated, and phenotype in the newborn); analytical characteristics of the screening test (eg, availability and features of the platform); and availability of health professionals experienced in diagnosis, treatment, and management to come to a recommended panel for screening. Disorders were scored and those with high scores, treatment availability, and well-understood natural history were included in the core panel. Other conditions with potential clinical significance but that did not fulfill the criteria to be in the core panels were included as secondary targets. Twenty-nine conditions were assigned to the core panel including nine organic acidemias, five fatty acids oxidation disorders, six aminoacidopathies, two other inborn errors of metabolism, two endocrinopathies, three hemoglobinopathies, hearing loss, and cystic fibrosis. The secondary targets included 25 disorders, most of which could be detected through the same analytes used for primary core disorders (**Table 1**).[1]

The Newborn Screening Saves Lives Act recommended the uniform screening panel with primary and secondary conditions. Shortly after this act, screening for the primary conditions on the recommended uniform screening panel was available to all infants born in the United States.[16] The Newborn Screening Saves Lives Act also gave the Advisory Committee on Heritable Disorders in Newborn and Children (ACHDNC) the authority to provide recommendations regarding potential additions to the recommended uniform screening. The committee follows an evidence-based process and on agreement by the committee, recommendations are made. Application to nominate a condition is submitted to the committee by physician, researchers, or advocacy groups. To date, the committee has approved recommendations for addition of screening for glycogen storage disease type II (Pompe disease), severe combined immunodeficiencies, T-cell-related lymphocyte deficiencies, and pulse oximetry for critical congenital heart diseases (see **Table 1**).[18] Although the ACHDNC makes recommendations of conditions to be added to newborn screening, the final decision of which disorders to be added to the newborn screening is usually made by each individual state. This decision is affected by population differences, technological competence, financial burden, and political environment that vary from state to state. Currently all US states provide testing for the original 29 recommended primary conditions. Some states have adopted testing for the additional disorders recommended by ACHDNC and some have included disorders not yet recommended by ACHDNC.[19]

OPTIMIZING NEWBORN SCREENING RESULTS

Tests in newborn screening are not designed to be diagnostic. Therefore, abnormal newborn screen results should prompt the initiation of further diagnostic testing. The follow-up diagnostic tests can confirm the disease suspected by the newborn screening and in this case the newborn screen is considered true positive. In contrast,

Table 1
Conditions included in newborn screening

Organic Acidemia	Fatty Acid Oxidation Defects	Aminoacidopathies	Other Inborn Errors of Metabolism	Endocrinopathies	Hemoglobinopathies	Others
ACMG core panel (29 primary conditions)						
1. Isovaleric acidemia 2. Propionic acidemia 3. Methylmalonic acidemia 4. Glutaric acidemia type I 5. Multiple carboxylase deficiency 6. Cobalamin A and B disorders 7. 3-Hydroxy-3-methyglutaric aciduria 8. 3-Methylcrotonyl-CoA carboxylase deficiency 9. β-Ketothiolase deficiency	1. Medium-chain acyl-CoA dehydrogenase deficiency 2. Very-long-chain acyl-CoA dehydrogenase deficiency 3. Long-chain hydroxyacyl-CoA dehydrogenase deficiency 4. Trifunctional protein deficiency 5. Carnitine uptake defect	1. Phenylketonuria 2. Maple syrup urine disease 3. Homocystinuria 4. CIT I 5. Argininosuccinic acidemia 6. TYR I	1. Biotinidase deficiency 2. Classic galactosemia	1. Congenital hypothyroidism 2. Congenital adrenal hyperplasia	1. Sickle cell anemia 2. Hemoglobin S/β-thalassemia 3. Hemoglobin S/C disease	1. Hearing loss 2. Cystic fibrosis

(*continued on next page*)

Table 1
(*continued*)

Organic Acidemia	Fatty Acid Oxidation Defects	Aminoacidopathies	Other Inborn Errors of Metabolism	Endocrinopathies	Hemoglobinopathies	Others
ACMG secondary targets (25 secondary conditions)						
1. Cobalamin C and D disorders 2. Malonic acidemia 3. Isobutyryl-CoA dehydrogenase deficiency 4. 2-Methyl-3-hydroxybutyric aciduria 5. 2-Methylbutyryl-CoA dehydrogenase deficiency 6. 3-Methylglutaconic aciduria	1. Short-chain acyl-CoA dehydrogenase deficiency 2. Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency 3. Glutaric acidemia type II 4. Medium-chain ketoacyl-CoA thiolase deficiency 5. Dienoyl-CoA reductase deficiency 6. CPT IA 7. CPT II 8. Carnitine/acylcarnitine translocase deficiency	1. Argininemia 2. CIT II 3. TYR II 4. TYR III 5. Hyperphenylalaninemia 6. Hypermethioninemia 7. Defects of biopterin cofactor biosynthesis 8. Defects of biopterin cofactor regeneration	1. Galactokinase deficiency 2. Galactose epimerase deficiency		1. Variant hemoglobinopathies (including hemoglobin E)	
Additional conditions recommended by ACHDNC						
1. Glycogen storage disease type II (Pompe disease) 2. Critical congenital heart diseases 3. Severe combined immunodeficiencies 4. T-cell-related lymphocyte deficiencies						

Abbreviations: ACHDNC, Advisory Committee on Heritable Disorders in Newborn and Children; ACMG, American College of Medical Genetics and Genomics; CIT, citrullinemia type I or II; CPT, carnitine palmitoyltransferase type I or II; TYR, tyrosinemia type I or II.

the follow-up diagnostic tests can be normal, excluding the disease suspected by the newborn screen and in this case the newborn screen is considered false positive. The newborn screen is considered false negative when an individual with the disease has a normal newborn screening result for that disease.[20]

In contrast to other clinical tests that have normal range out of which the test is reported to be abnormal, newborn screening tests have cutoff values that are used to determine whether tests are normal (not requiring further testing) or abnormal (requiring further testing). The cutoff value could be either above (high) or below (low) the normal population. The high target value is set in the interval between the 99th percentile of the normal population and the lowest 5th percentile of the affected individual range. However, the low target value is set in the interval between the highest 99th percentile of affected individual range and the 1st percentile of the normal population. Each screening program determines where to set cutoff values for each test aiming for low false-positive and false-negative rates. However, when there is overlap between normal and affected individuals, newborn screening programs typically elect to set the cutoff values that minimize the false-negative rates, which may lead to a higher false-positive rate.[20]

Because abnormal newborn screening results not only carry the consequences of burden of follow-up clinical evaluation and its cost, but can also cause significant parental anxiety,[21–23] efforts have been made to optimize the cutoff points to minimize the false-positive rate while maintaining low false-negative rate. Traditionally, each newborn screening program set its own cutoff values based mainly on normal population results. Once cutoff values selected in this manner are implemented, negative feedback from the follow-up system (too many false positives) or the dreaded occurrence of a false negative case may lead to abrupt changes on the cutoff. Significant improvement in cutoff value determination was achieved by an international collaboration that collected millions of normal and true-positive newborn screening results and determined the percentiles for both populations. The cutoff target ranges of analytes were then defined as the interval between selected percentiles of the two populations.[24]

Other than cutoff points optimization, different approaches have been implemented to reduce the false-positive and -negative rates. First, secondary analytes have been typically used to improve sensitivity and reduce false-positive rate. Second, for some disorders with abnormal results on initial screen, a reflex to a more specific test is done to support a diagnosis and decrease the false-positive rate for a particular test. This second-tier reflex testing can be a measurement of additional metabolites (eg, steroid profiling in congenital adrenal hyperplasia and succinylacetone in tyrosinemia type I), or it can be DNA sequencing to detect common pathogenic variants (eg, cystic fibrosis, medium-chain acyl-CoA dehydrogenase deficiency, and galactosemia).[25–27] Third, a computational approach, that has proved its utility, depends on multivariate pattern recognition software that generates tools integrating multiple clinically significant results into a single score. Retrospective evaluation of past cases suggested that these tools could have avoided significant percentage of the false-positive and could have prevented most of known false-negative events.[28]

LOGISTICS AND FOLLOW-UP

Newborn screening is a system rather than an event in which a test is simply performed. This system includes preanalytical, testing, and postanalytical phases. The preanalytical phase typically takes place at the birth hospital where demographic data are collected and documented, blood sampling is obtained, and filter paper cards

are shipped. The testing phase occurs at designated department of health laboratories and includes samples preparation, test conduction, results interpretation, and report issuing. The final and most important phase is the postanalytical phase where results for newborn screening requiring further testing are communicated and confirmed, treatment is initiated, and long-term follow-up is monitored. During this entire process, timeliness of sample transport, test performance, results transmission, and availability of confirmatory testing and treatment are critical to ensure the success of newborn screening programs. Continuous quality assurance for the performed tests, easy access to health care, and system evaluation are also critical for the success of this system.[3,29]

Good communication is an essential part of any successful newborn screening program. Department of health laboratories performing the newborn screening tests, primary health care providers, metabolic centers, and families should all communicate effectively to ensure a timely evaluation and management of newborns whose newborn screening test indicates that further testing is required. Typically, the laboratory reports newborn screening results to the primary health care provider listed on the newborn screening card, who will subsequently notify the family of the results and perform the recommended follow-up evaluation, or refer the infant to a metabolic specialist. In certain urgent cases, the laboratory may also contact the metabolic specialist and the family directly.[21,30] Abnormal newborn screening results requiring further testing do not only carry the consequences of burden of follow-up clinical evaluation and its cost, but can also cause significant parental anxiety. Expectedly, parents who are well-informed by their primary care physician typically feel less stress about the results compared with parents who are not educated. Therefore, parents should be appropriately counseled with regard to the nature of newborn screening, the results in their child, and the possible clinical implication. Particularly, it should be emphasized that results of newborn screening indicate that the infant may be at risk to be affected with a condition, and further testing is needed, but newborn screening results do not indicate definitive diagnoses.[2,21–23]

FACTORS AFFECTING NEWBORN SCREENING RESULTS

Several factors can affect the newborn screening results including the timing of blood sampling. Newborn screening test needs to be done between 24 and 48 hours of life. Some states require a second newborn screen that is usually performed between 7 and 14 days of life. It is important to adhere to the recommend timeframe for obtaining newborn screening tests because the cutoff values for screening analytes are set to reflect values expected at this age range. Obtaining a newborn screening earlier than 24 hours of life can decrease the sensitivity of testing. Beyond the neonatal period, there are no well-established cutoff values.[31,32] In addition, the level of analytes measured in newborn screening is affected by nutrition (parental nutrition vs infant formula or breastfeeding), underlying sickness, gestational age, and birth weight.[33] All of these factors should be taken into consideration when interpreting the results and newborn screening programs typically include this information in the required demographic information of newborn being screened.

For sick newborns in neonatal intensive care units, blood transfusion may affect certain newborn screening results because of the mixture of the transfused blood in the neonate's bloodstream. Newborn screening enzyme tests for galactosemia and biotinidase deficiency, and hemoglobinopathies do not produce accurate results after transfusion; therefore, to permit screening for these conditions it is generally recommended to collect the newborn screening samples before the transfusion starts, if

possible, even if this takes place earlier than the usual time frame for obtaining the newborn screening. If the newborn screening is obtained at an earlier time because transfusion is needed, the neonate should still have another newborn screening for detection of disorders not affected by transfusion at the standard time frames.[3] In addition, the newborn screening enzyme tests for biotinidase deficiency and galactosemia is affected by environmental factors, such as temperature and humidity.[34]

Finally, a mother with an inborn error of metabolism may transmit abnormal metabolites to her offspring, confounding the infant's newborn screening results. Many cases of abnormal newborn screening for healthy infants of women with carnitine uptake defect, very-long-chain acyl-CoA dehydrogenase deficiency, 3-methylcrotonyl-CoA carboxylase deficiency, and others have been described.[35–37]

CONFIRMATION OF NEWBORN SCREENING RESULTS

Many of the conditions tested in the newborn screening are rare; therefore, the primary health care provider may not be familiar with the nature of the condition and the diagnostic work-up that needs to be done. A set of action (ACT) sheets that provides the information and guidelines of care for each condition in the newborn screen has been developed collaboratively by the American College of Medical Genetics and Genomics and the American Academy of Pediatrics.[38] For most inborn errors of metabolism, the initial follow-up testing involves the determination of different metabolites in urine and blood using such tests as plasma amino acids, urine organic acids, and acylcarnitine profile. When the results of the initial follow-up tests are abnormal or equivocal, further testing including enzyme assay and gene sequencing may be used for definitive diagnosis (**Table 2**).

FUTURE DIRECTIONS

Newborn screening will continue to expand by the addition of disorders for which early intervention can significantly modify the outcome. This expansion is driven by the development of new therapies for larger number of inborn errors of metabolism and the advancement in testing methodologies.

With the advancement in enzyme-replacement therapy development for lysosomal storage disorders, these diseases have been considered for inclusion in newborn screening.[39–41] A few states have begun screening for Krabbe, adrenoleukodystorphy, Fabry, Gaucher, Niemann-Pick, and Hurler, although these diseases have not yet been recommended by ACHDNC because of the lack of evidence of the efficacy of screening.[42,43] Besides storage diseases, other conditions have been evaluated for inclusion in newborn screening, such as guanidinoacetoacetate methyltransferase deficiency, cerebrotendinous xanthomatosis, biliary atresia, and Duchenne muscular dystrophy.[44–47]

Although advancements in the utility of tandem mass spectrometry methodology have allowed for expanding the newborn screening,[48] other methodologies have been also considered. With the advancement of next-generation sequencing technology targeted gene panel and whole exome sequencing have been considered as first-tier or second-tier genetic testing in newborn screening. However, the high costs, prolonged turnaround time, and the detection of variants of uncertain clinical significance are currently significant limitations for the use of this methodology for newborn screening.[49–53] In contrast to biochemical profiling, molecular testing are time-independent and therefore can be performed earlier than 24 hours of life. In fact, molecular testing can be performed even before birth during fetal life if samples can be obtained noninvasively. Currently, great advancements have been achieved in

Table 2
Follow-up testing for abnormal newborn screening

		Follow-up Tests										
		Initial Testing										
	Abnormal Newborn Screen Analytes	Plasma Amino Acids	Urine Organic Acids	Acylcarnitine Profile	Total and Free Carnitine	Urine Acylglycines	Urine Orotic Acid	Total Plasma Homocysteine	Plasma Phenylalanine	Plasma Methylmalonic Acid	Urine Succinylacetone	Additional Confirmatory Testing
Organic acidemia												
Propionic acidemia	↑ C3 (propionylcarnitine)	—	X	X	—	X	—	—	—	—	—	Enzyme assay in fibroblast or leukocytes; *PCCA, PCCB* gene sequencing
Methylmalonic acidemia	↑ C3	—	X	X	—	X	—	—	—	X	—	Enzyme assay in fibroblast; *MUT* gene sequencing
Cobalamin disorders	↑ C3	—	X	X	—	X	—	X	—	X	—	Cobalamin complementation studies in fibroblast; *MMAA, MMAB, MMACHC* gene sequencing
Malonic acidemia	↑ C3-DC (malonylcarnitine)	—	X	X	—	—	—	—	—	X	—	*MLYCD* gene sequencing
Isobutyryl-CoA dehydrogenase deficiency	↑ C4 (isobutyrylcarnitine)	—	—	X	—	X	—	—	—	—	—	*ACAD8* gene sequencing
Isovaleric acidemia	↑ C5 (isovalerylcarnitine/ methylbutyrylcarnitine)	—	X	X	—	X	—	—	—	—	—	*IVD* gene sequencing
2-Methylbutyryl-CoA dehydrogenase deficiency	↑ C5	—	X	X	—	X	—	—	—	—	—	*ACADSB* gene sequencing
Glutaric acidemia type I	↑ C5-DC (glutarylcarnitine)	—	X	X	—	—	—	—	—	—	—	Enzyme assay in fibroblast; *GDCH* gene sequencing
Biotinidase deficiency	↑ C5-OH (hydroxy-isovalerylcarnitine/ methyl-hydroxybutyrylcarnitine)	—	X	X	—	X	—	—	—	—	—	Biotinidase enzyme assay in serum; *BTD* gene sequencing
Multiple carboxylase deficiency	↑ C5-OH	—	X	X	—	X	—	—	—	—	—	Enzyme testing in fibroblast, leukocytes; *HLCS* gene sequencing

3-Methylcrotonyl-CoA carboxylase deficiency	↑ C5-OH	—	X	X	—	X	—	—	—	—	—	Enzyme testing in fibroblast, leukocytes; *MCCC1*, *MCCC2* gene sequencing
Hydroxy-3-methyglutaric aciduria	↑ C5-OH	—	X	X	—	X	—	—	—	—	—	*HMGCL* gene sequencing
3-Methylglutaconic aciduria	↑ C5-OH	—	X	X	—	X	—	—	—	—	—	Enzyme assay in fibroblasts; *AUH* gene sequencing
β-Ketothiolase deficiency	↑ C5-OH	—	X	X	—	—	—	—	—	—	—	Enzyme assay in fibroblasts; *ACAT1* gene sequencing
2-Methyl-3-hydroxybutyric aciduria	↑ C5-OH	—	X	X	—	—	—	—	—	—	—	Enzyme assay in fibroblasts; *HSD17B10* gene sequencing
Fatty acid oxidation defects												
Carnitine uptake defect	↓ C0 (free carnitine)	—	—	—	X	—	—	—	—	—	—	Carnitine transport assay in fibroblasts; *OCTN2* gene sequencing
CPT IA	↑ C0	—	—	X	X	—	—	—	—	—	—	CPT assay in fibroblasts; *CPT1A* gene sequencing
Short-chain acyl-CoA dehydrogenase deficiency	↑ C4 (butyrylcarnitine)	—	X	X	—	X	—	—	—	—	—	*ACADS* gene sequencing
Glutaric acidemia type II	↑ C4	—	X	X	—	X	—	—	—	—	—	*ETFA*, *ETFB*, *ETFDH* gene sequencing
Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency	C4-OH (3-hydroxy-butyrylcarnitine)	—	X	X	—	—	—	—	—	—	—	*HADH* gene sequencing
Medium-chain acyl-CoA dehydrogenase deficiency	↑ C8 (octanoylcarnitine)	—	X	X	—	X	—	—	—	—	—	*ACADM* gene sequencing
Very-long-chain acyl-CoA dehydrogenase deficiency	↑ C14:1 (tetradecenoylcarntine)	—	—	X	—	—	—	—	—	—	—	*ACADVL* gene sequencing
CPT II	↑ C16 (hexadecanoylcarnitine)	—	—	X	—	—	—	—	—	—	—	*CPT2* gene sequencing

(*continued on next page*)

Table 2
(*continued*)

		Follow-up Tests										
		Initial Testing										
	Abnormal Newborn Screen Analytes	Plasma Amino Acids	Urine Organic Acids	Acylcarnitine Profile	Total and Free Carnitine	Urine Acylglycines	Urine Orotic Acid	Total Plasma Homocysteine	Plasma Phenylalanine	Plasma Methylmalonic Acid	Urine Succinylacetone	Additional Confirmatory Testing
Carnitine/ acylcarnitine translocase deficiency	↑ C16	—	—	X	—	—	—	—	—	—	—	*SLC25A20* gene sequencing
Long-chain hydroxyacyl-CoA dehydrogenase deficiency	↑ C16-OH (hydroxyl-hexadecanoylcarnitine)	—	—	X	—	—	—	—	—	—	—	*HADHA* gene sequencing
Trifunctional protein deficiency	↑ C16-OH	—	—	X	—	—	—	—	—	—	—	*HADHA*, *HADHB* gene sequencing
Aminoacidopathies												
Phenylketonuria	↑ Phenylalanine	—	—	—	—	—	—	—	X	—	—	*PAH* gene sequencing
Defects of biopterin cofactor metabolism	↑ Phenylalanine	—	—	—	—	—	—	—	X	—	—	Urine pterins; CSF neurotransmitters; enzyme assay in erythrocytes; *GCH1*, *PTS*, or *QDPR* gene sequencing
Maple syrup urine disease	↑ Leucine	X	X	—	—	—	—	—	—	—	—	Enzyme assay in lymphoblast or fibroblast; *BCKDHA*, *BCKDHB*, *DBT* gene sequencing
Homocystinuria	↑ Methionine	X	—	—	—	—	—	X	—	—	—	*CBS* gene sequencing
Hypermethioninemia	↑ Methionine	X	—	—	—	—	—	X	—	—	—	Plasma S-adenosylhomocysteine and S-adenosylmethionine; *MAT1A* gene sequencing
Argininemia	↑ Arginine	X	—	—	—	—	X	—	—	—	—	Enzyme assay in erythrocytes or liver; *ARG1* gene sequencing

Argininosuccinic acidemia	↑ Citrulline	X	—	—	—	—	X	—	—	—	—	Enzyme assay in erythrocytes, fibroblast, or liver; *ASL* gene sequencing
CIT I	↑ Citrulline	X	—	—	—	—	X	—	—	—	—	Enzyme assay in fibroblast or liver; *ASS1* gene sequencing
CIT II	↑ Citrulline	X	—	—	—	—	—	—	—	—	—	*SLC25A13* gene sequencing
TYR I	↑ Succinylacetone	X	X	—	—	—	—	—	—	—	X	*FAH* gene sequencing
TYR II	↑ Tyrosine	X	X	—	—	—	—	—	—	—	—	*TAT* gene sequencing
TYR III	↑ Tyrosine	X	X	—	—	—	—	—	—	—	—	*HPD* gene sequencing
Endocrinopathies												
Congenital hypothyroidism	↑ Thyroid-stimulating hormone	Serum free T4 and thyroid-stimulating hormone										—
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	↑ 17-OHP (17-hydroxyprogesterone)	Serum 17-OHP, electrolytes, and glucose										Adrenocorticotropic hormone stimulation test; steroid profile; *CYP21A2* gene sequencing
Hemoglobinopathies												
Sickle cell anemia	+HbS	CBC, reticulocyte count, and hemoglobin electrophoresis										*HBB* gene sequencing
Hemoglobin S/β-thalassemia	+HbS	CBC, reticulocyte count, and hemoglobin electrophoresis										*HBB* gene sequencing
Hemoglobin S/C disease	+HbS	CBC, reticulocyte count, and hemoglobin electrophoresis										*HBB* gene sequencing
Cystic fibrosis	↑ Immunoreactive trypsinogen	Sweat chloride and *CFTR* gene sequencing										—

Abbreviations: CBC, complete blood count; CIT, citrullinemia type I or II; CPT, carnitine palmitoyltransferase type I or II; TYR, tyrosinemia type I or II.

noninvasive prenatal diagnosis through the extraction of cell-free fetal DNA from maternal blood for the detection of aneuploidy and genome-wide copy number variation.[54] With more advancement in this field, reliable sequencing data can be obtained from such methodology, which can open the doors for the potentials of doing molecular screening during fetal life.

Another approach that can be potentially used in newborn screening is global metabolomic profiling, which is a mass spectrometry–based method that allows the detection of huge numbers of small molecules in body fluids, and can be applied for the screening of a large number of inborn errors of metabolism.[55–57]

An integrated approach combining the molecular data from next-generation sequencing and biochemical data from global metabolimics can potentially produce robust results. The metabolic profiles can provide a functional readout to assess the penetrance of gene mutations identified by next-generation sequencing. Conversely, metabolic abnormalities can uncover potential damaging mutations that were previously unappreciated by sequencing.[58] Although such an approach has not been considered for newborn screening, it can be of potential utility in the future with more advancement in technologies.

Therefore, further advancement in technology is expected to allow continuous expansion of conditions detected by newborn screening with reduction in cost, shorter turnaround time, and more accurate results with low false-positive and false-negative results.

SUMMARY

Newborn screening aims to achieve early diagnosis of treatable disorders to allow for early initiation of medical care to prevent or reduce significant morbidity and mortality related to these disorders. Many of the conditions tested in newborn screening are inborn errors of metabolism; however, it also tests for some other endocrine, hematologic, and immunologic diseases. Newborn screening tests are not diagnostic. Therefore, abnormal newborn screen results require follow-up tests to confirm or exclude the suspected diagnosis. Further advancement in technology is expected to allow continuous expansion of newborn screening.

REFERENCES

1. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system–executive summary. Pediatrics 2006;117(5 Pt 2):S296–307.
2. Almannai M, Marom R, Sutton VR. Newborn screening: a review of history, recent advancements, and future perspectives in the era of next generation sequencing. Curr Opin Pediatr 2016;28(6):694–9.
3. Berry SA. Newborn screening. Clin Perinatol 2015;42(2):441–53, x.
4. Grosse SD. Does newborn screening save money? The difference between cost-effective and cost-saving interventions. J Pediatr 2005;146(2):168–70.
5. Pena LDM, van Calcar SC, Hansen J, et al. Outcomes and genotype-phenotype correlations in 52 individuals with VLCAD deficiency diagnosed by NBS and enrolled in the IBEM-IS database. Mol Genet Metab 2016;118(4):272–81.
6. Posset R, Garcia-Cazorla A, Valayannopoulos V, et al. Age at disease onset and peak ammonium level rather than interventional variables predict the neurological outcome in urea cycle disorders. J Inherit Metab Dis 2016;39(5):661–72.

7. Ding Y, Thompson JD, Kobrynski L, et al. Cost-effectiveness/cost-benefit analysis of newborn screening for severe combined immune deficiency in Washington state. J Pediatr 2016;172:127–35.
8. Castellani C, Massie J, Sontag M, et al. Newborn screening for cystic fibrosis. Lancet Respir Med 2016;4(8):653–61.
9. Chaturvedi S, DeBaun MR. Evolution of sickle cell disease from a life-threatening disease of children to a chronic disease of adults: the last 40 years. Am J Hematol 2016;91(1):5–14.
10. Bickel H, Gerrard J, Hickmans EM. Influence of phenylalanine intake on phenylketonuria. Lancet 1953;265(6790):812–3.
11. Horner FA, Streamer CW. Effect of a phenylalanine-restricted diet on patients with phenylketonuria; clinical observations in three cases. J Am Med Assoc 1956; 161(17):1628–30.
12. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338–43.
13. Heard GS, Secor McVoy JR, Wolf B. A screening method for biotinidase deficiency in newborns. Clin Chem 1984;30(1):125–7.
14. Beutler E, Baluda M, Donnell GN. A new method for the detection of galactoxemia and its carrier state. J Lab Clin Med 1964;64:694–705.
15. Millington DS, Kodo N, Norwood DL, et al. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. J Inherit Metab Dis 1990;13(3):321–4.
16. Public Law 110-204-Newborn screening saves lives act of 2007. Available at: https://www.gpo.gov/fdsys/pkg/PLAW-110publ204/content-detail.html. Accessed November 15, 2016.
17. Wilson J, Jungner J. Principles and practices of screening for disease. Geneva (Switzerland): World Health Organization; Public Health Papers; 1968. p. 34.
18. Recommended Uniform Screening Panel. Available at: http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel/index.html. Accessed November 16, 2016.
19. Screening Programs | National Newborn Screening and Global Resource Center. Available at: http://genes-r-us.uthscsa.edu/screening. Accessed November 15, 2016.
20. Sutton VR, Graham BH. Newborn screening for inborn errors of metabolism, introduction and approaches for confirmation. In: Lee B, Scaglia F, editors. Inborn errors of metabolism, from neonatal screening to metabolic pathways. New York: Oxford University Press; 2015. p. 3–34.
21. Davis TC, Humiston SG, Arnold CL, et al. Recommendations for effective newborn screening communication: results of focus groups with parents, providers, and experts. Pediatrics 2006;117(5 Pt 2):S326–40.
22. Gurian EA, Kinnamon DD, Henry JJ, et al. Expanded newborn screening for biochemical disorders: the effect of a false-positive result. Pediatrics 2006; 117(6):1915–21.
23. Schmidt JL, Castellanos-Brown K, Childress S, et al. The impact of false-positive newborn screening results on families: a qualitative study. Genet Med 2012;14(1): 76–80.
24. McHugh DMS, Cameron CA, Abdenur JE, et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genet Med 2011;13(3):230–54.

25. Lacey JM, Minutti CZ, Magera MJ, et al. Improved specificity of newborn screening for congenital adrenal hyperplasia by second-tier steroid profiling using tandem mass spectrometry. Clin Chem 2004;50(3):621–5.
26. Magera MJ, Gunawardena ND, Hahn SH, et al. Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. Mol Genet Metab 2006;88(1):16–21.
27. Baker MW, Groose M, Hoffman G, et al. Optimal DNA tier for the IRT/DNA algorithm determined by CFTR mutation results over 14 years of newborn screening. J Cyst Fibros 2011;10(4):278–81.
28. Marquardt G, Currier R, McHugh DMS, et al. Enhanced interpretation of newborn screening results without analyte cutoff values. Genet Med 2012;14(7):648–55.
29. Centers for Disease Control and Prevention (CDC). CDC grand rounds: newborn screening and improved outcomes. MMWR Morb Mortal Wkly Rep 2012;61(21):390–3.
30. Kim S, Lloyd-Puryear MA, Tonniges TF. Examination of the communication practices between state newborn screening programs and the medical home. Pediatrics 2003;111(2):E120–6.
31. Walter JH, Patterson A, Till J, et al. Bloodspot acylcarnitine and amino acid analysis in cord blood samples: efficacy and reference data from a large cohort study. J Inherit Metab Dis 2009;32(1):95–101.
32. Tang H, Feuchtbaum L, Neogi P, et al. Damaged goods?: an empirical cohort study of blood specimens collected 12 to 23 hours after birth in newborn screening in California. Genet Med 2016;18(3):259–64.
33. Clark RH, Kelleher AS, Chace DH, et al. Gestational age and age at sampling influence metabolic profiles in premature infants. Pediatrics 2014;134(1):e37–46.
34. Adam BW, Hall EM, Sternberg M, et al. The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. Clin Biochem 2011;44(17–18):1445–50.
35. McGoey RR, Marble M. Positive newborn screen in a normal infant of a mother with asymptomatic very long-chain Acyl-CoA dehydrogenase deficiency. J Pediatr 2011;158(6):1031–2.
36. El-Hattab AW, Li F-Y, Shen J, et al. Maternal systemic primary carnitine deficiency uncovered by newborn screening: clinical, biochemical, and molecular aspects. Genet Med 2010;12(1):19–24.
37. Kör D, Mungan NÖ, Yılmaz BŞ, et al. An asymptomatic mother diagnosed with 3-methylcrotonyl-CoA carboxylase deficiency after newborn screening. J Pediatr Endocrinol Metab 2015;28(5–6):669–71.
38. Information NC for B, Pike USNL of M 8600 R, MD B, USA 20894. Newborn screening ACT sheets and confirmatory algorithms. American College of Medical Genetics. 2001. Available at: https://www.ncbi.nlm.nih.gov/books/NBK55827/. Accessed November 16, 2016.
39. Kumar AB, Masi S, Ghomashchi F, et al. Tandem mass spectrometry has a larger analytical range than fluorescence assays of lysosomal enzymes: application to newborn screening and diagnosis of mucopolysaccharidoses types II, IVA, and VI. Clin Chem 2015;61(11):1363–71.
40. Matern D, Gavrilov D, Oglesbee D, et al. Newborn screening for lysosomal storage disorders. Semin Perinatol 2015;39(3):206–16.
41. Elliott S, Buroker N, Cournoyer JJ, et al. Pilot study of newborn screening for six lysosomal storage diseases using tandem mass spectrometry. Mol Genet Metab 2016;118(4):304–9.

42. Wasserstein MP, Andriola M, Arnold G, et al. Clinical outcomes of children with abnormal newborn screening results for Krabbe disease in New York State. Genet Med 2016;18(12):1235–43.
43. Kemper AR, Brosco J, Comeau AM, et al. Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation. Genet Med 2017;19(1):121–6.
44. Wang KS. Section on surgery, committee on fetus and newborn, childhood liver disease research network. Newborn screening for biliary atresia. Pediatrics 2015;136(6):e1663–9.
45. Bleyle L, Huidekoper HH, Vaz FM, et al. Update on newborn dried bloodspot testing for cerebrotendinous xanthomatosis: an available high-throughput liquid-chromatography tandem mass spectrometry method. Mol Genet Metab Rep 2016;7:11–5.
46. Gatheridge MA, Kwon JM, Mendell JM, et al. Identifying non-Duchenne muscular dystrophy-positive and false negative results in prior Duchenne muscular dystrophy newborn screening programs: a review. JAMA Neurol 2016;73(1):111–6.
47. Pasquali M, Schwarz E, Jensen M, et al. Feasibility of newborn screening for guanidinoacetate methyltransferase (GAMT) deficiency. J Inherit Metab Dis 2014;37(2):231–6.
48. Ombrone D, Giocaliere E, Forni G, et al. Expanded newborn screening by mass spectrometry: new tests, future perspectives. Mass Spectrom Rev 2016;35(1):71–84.
49. Bhattacharjee A, Sokolsky T, Wyman SK, et al. Development of DNA confirmatory and high-risk diagnostic testing for newborns using targeted next-generation DNA sequencing. Genet Med 2015;17(5):337–47.
50. Poulsen JB, Lescai F, Grove J, et al. High-quality exome sequencing of whole-genome amplified neonatal dried blood spot DNA. PLoS One 2016;11(4):e0153253.
51. Botkin JR, Rothwell E. Whole genome sequencing and newborn screening. Curr Genet Med Rep 2016;4(1):1–6.
52. Wu C-C, Tsai C-H, Hung C-C, et al. Newborn genetic screening for hearing impairment: a population-based longitudinal study. Genet Med 2017;19(1):6–12.
53. Narravula A, Garber KB, Askree SH, et al. Variants of uncertain significance in newborn screening disorders: implications for large-scale genomic sequencing. Genet Med 2017;19(1):77–82.
54. Kølvraa S, Singh R, Normand EA, et al. Genome-wide copy number analysis on DNA from fetal cells isolated from the blood of pregnant women. Prenat Diagn 2016;36(12):1127–34.
55. Goodacre R, Vaidyanathan S, Dunn WB, et al. Metabolomics by numbers: acquiring and understanding global metabolite data. Trends Biotechnol 2004;22(5):245–52.
56. Miller MJ, Kennedy AD, Eckhart AD, et al. Untargeted metabolomic analysis for the clinical screening of inborn errors of metabolism. J Inherit Metab Dis 2015;38(6):1029–39.
57. Dénes J, Szabó E, Robinette SL, et al. Metabonomics of newborn screening dried blood spot samples: a novel approach in the screening and diagnostics of inborn errors of metabolism. Anal Chem 2012;84(22):10113–20.
58. Guo L, Milburn MV, Ryals JA, et al. Plasma metabolomic profiles enhance precision medicine for volunteers of normal health. Proc Natl Acad Sci U S A 2015;112(35):E4901–10.