Newborn Screening: An Overview

EILEEN CARREIRO-LEWANDOWSKI

The ethical considerations and the criteria for inclusion of a test to a newborn screening program have remained constant since testing began in the 1960s. Does the test identify a treatable disorder with significant incidence to pose a public health risk and warrant testing all babies in that state or territory? Technological advances in testing, particularly with the improvement of tandem mass spectrometry techniques and the advent of DNA testing for the specific gene mutations, have expanded our understanding of many inherited metabolic diseases. These mostly autosomal recessive disorders went under-diagnosed by the medical community for many years. This was partly due to the notion that the incidence of inherited metabolic diseases was quite rare and that many so-called birth defects, or unexplained infant deaths, were not associated with any known metabolic disorders.

Public health departments, as part of their newborn health programs, offer some newborn screening to all infants born within their jurisdiction. Two tests, those for phenylketonuria (PKU) and congenital hypothyroidism are universally mandated (51/51 juristictions). The next highest frequency tests are for galactosemia and sickle cell disease (50/51), with up to thirty tests available in some states. However, the authority as to which tests are included resides with the local state government, either as a matter of law or as a matter for the public health department. As these matters become more complex, many public health officials and pediatric healthcare practitioners urge the Federal government to become involved and develop national guidelines in an effort to streamline the process and decrease the existing inconsistencies between states.

For many laboratorians, the collection of newborn screening blood spot samples is the extent of their involvement in newborn screening programs. The many facets of these programs, the status of newborn screening in the United States, and the incidence and description of selected inherited disorders are explored.

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ABBREVIATIONS: BIO = biotinidase deficiency; CAH = congenital adrenal hyperplasia; CF = cystic fibrosis; CH = congenital hypothyroidism; FOD = fatty acid oxidation disorders which include MCAD (medium chain acyl-CoA dehydrogenase deficiency); GAL = galactosemia; HCY = homocystinuria; MS/MS = tandem mass spectrometry; MSUD = maple syrup urine disease; OAA = other amino acidemias including tyrosinemia types I and II; OAD = organic acidemias; PKU = phenylalaninemia; SCD = sickle cell disease.

INDEX TERMS: newborn diseases; newborn testing.

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LEARNING OBJECTIVES

At the end of the article, the learner will be able to:

1. Identify the ethical issues surrounding newborn screening.
2. Discuss the impact a lack of nationally adopted standards has on newborn screening.
3. Describe the criteria used for incorporation of a test in a newborn screening program.
4. Discuss the impact of technological advances on newborn screening.
5. Explain the principle of tandem-mass spectrometry.
6. Identify the pre- and post-analytical variables related to newborn screen testing.
7. Define the deficiency and discuss the physiologic implications associated with the inherited metabolic diseases presented.
8. Discuss the relative incidence of the 12 inherited disorders described.
9. Compare voluntary and mandatory screening.

10. List the factors that historically impeded early diagnosis of inherited metabolic diseases.
11. List the two disorders screened by all U.S. states.
12. List the 15 states that offer testing (at least MCAD) for fatty acid oxidation disorders.

The idea of newborn screening began with testing for an inherited disorder, phenylketonuria, in 1962. Advances in medical research and analytical techniques has lead to the discovery and ability to test for many inherited metabolic diseases and other birth disorders. The more recent (1990s) development of newborn screening methods using tandem mass spectrometry (MS/MS) allows for a wide array of amino, organic, and fatty acid inherited disorders to be profiled using a single blood sample. Further development of allele-specific molecular diagnostics will only cause this issue to continue to evolve. The decision as to which tests to include in mandated newborn screening programs, that is testing performed as a matter of state law on all babies born in that jurisdiction or according to state guidelines in high risk populations, is currently determined by each state, and varies from state to state. This means that no uniformity exists, and the testing done on a newborn in one state, may not match the screening tests performed in a neighboring state. The American Academy of Pediatrics has made recommendations to the federal government to support a national advisory committee to develop uniform, national standards so that each state can properly evaluate which tests should be included in a newborn screening program.[1] The underlying ethical questions only increase when considering the many other issues related to newborn screening.

Incorporation of a test into a screening program traditionally satisfied certain criteria. The test should identify a treatable disorder and any medical intervention should improve the affected individual's quality of life. That is, the identification of the genetic disorder is of benefit to the child. The disorder should be sufficiently common in a specific or the general population that it constitutes a health risk if left untreated. Other criteria involve the test method's analytical and diagnostic capabilities. They must be of sufficient quality to provide reliable results in a given population. If the incidence of the disease is low, is it worth screening all newborns or just selected high-risk groups? Should the frequency of positive cases justify inclusion (or exclusion) of a test in the newborn screening program? What of the technological advances allowing for the early identification of untreatable diseases, do these disorders qualify as a public health risk, justifying mandated testing? Should treatment options be a relevant consideration for inclusion in a newborn screening program? Who defines quality of life or benefit? Who decides?

Universal screening (all infants born in that state or territory) exists for PKU and congenital hypothyroidism. Testing for galactosemia is performed in all states except Washington, and 43 states plus the District of Columbia screen all newborns for sickle cell disease. Additionally, six states test for sickle cell disease as part of a selected population or pilot program.[2] Idaho does not offer any screening for sickle cell disease, yet the risk of sickle cell disease is the same as in other states.[3] The case of sickle cell disease testing in Idaho illustrates an interesting scenario easily applied to any inherited disorder. The prevalence of sickle cell disease varies between states due to differences in the ethnic populations located in each geographical area. This fact serves as the rationale for selected states to exclude sickle cell disease, as well as other disorders, in their mandated newborn screening program. In fact, some states have eliminated certain tests because of the infrequency of infants found with the inherited disorder. However, the likelihood of an African-American infant born in Idaho of suffering from sickle cell disease is the same as in other states but an affected infant could suffer grave consequences because of a lack of testing. Inequities and inconsistencies in newborn screening programs between states reflect the decision-making concerns of state policymakers and public health managers. Factors influencing these decisions include the local values of parents, recommendations from professional groups, scientific/medical expertise of lawmakers, and budgetary considerations and restrictions, plus concerns regarding privacy issues. The methods used to make these decisions need to move from the domain of various recommendations and opinions to those based on more empirical data gleaned from analytical studies, clinical trials, research, and collaboration of information between state and state agencies. Some states have responded with pilot programs as a mechanism to collect this data and as a means to explore expanding test menus.

Another basic tenet is that the testing must be affordable and have a sufficiently short turn-around-time so that treatment occurs in a timely fashion before the infant suffers harm. Many state budgetary constraints prevent expansion of newborn screening programs simply because the funding to do so is unavailable. New Jersey recently passed legislation to expand its testing services, but inadequate funding has stalled implementation. If a test is part of a state mandated program, all babies are tested without regard to ability to pay for this service. A newborn screening program consists of five elements: 1) screening; 2) short-term follow-up; 3) diagnosis; 4) treatment and management; and 5) program evaluation and quality assurance.[4] A combination of newborn screening fees, general public health funding, and reliance on third-party payers helps fund the cost of newborn screening programs. Many, but not all (approximately 30) states collect a fee for testing, which include both laboratory and other program services. Of these, 17 states financed more comprehensive services that included follow-up and treatment costs.[5] Fees may be billed as part of the parents' medical insurance for allowed newborn care medical coverage, directly to the parents without consideration of healthcare benefits, directly to the primary healthcare professional as part of their fees or services, or as a portion of the delivery charges. Testing fees do not necessarily cover all costs of the newborn screening program. Adequate funding must cover the costs associated with all aspects of the screening program—

pre-analytical, analytical, and post-analytical—from sample collection, transportation, testing, repeat and confirmatory sampling and testing, and parent and healthcare practitioner education to reporting of results. Additionally, the screening program must also address the needs of its population by providing counseling and a system of referral to appropriate medical specialists. Not all children and their parents have health coverage or the means to purchase needed treatment. This is particularly true when costly special formulas, neurodevelopment assessments, therapies, and psycho-social services may not be funded through the patient's health plan or only for a limited amount of time. In some managed care organizations, the plan requires utilization of in-network professionals without regard to their expertise.

The extent of communication associated with a newborn screening program can be complex. While newborn screen samples may be obtained from a birthing center, mid-wife in the residence, or hospital, most of the testing is performed in private, state, or regional public health laboratories, separate from the birth facility. In the event of repeat sampling and necessary follow-up, the physician ordering the test may not always serve as the infant's primary healthcare practitioner, particularly in situations where the mother hasn't identified a pediatrician or a clinic name, or uses a clinic or medical practice where more than one healthcare provider may be in charge of care. The policies surrounding the reporting of results vary widely between states. Inconsistencies exist between states as to the law concerning the reporting authority and/or recipient. In some states, results are provided to the infant's place of birth, the physician present at birth, and the pediatrician while others only provide reports to the pediatrician, if positive. In these states there's an assumption of a negative test, when in fact, a baby, for example discharged before 24 hours of age, may have been overlooked. This makes reporting of results and any necessary follow-up extremely difficult.[6] In an attempt to streamline this process and minimize errors, the American Academy of Pediatrics initially suggested that the parent should assign a 'medical home' to each child at birth or a short time later.[7] This concept may not only apply to newborn screening but also include immunization records, vision and hearing test results, and other medically important facts. Consensus as to its definition, ramifications such as privacy issues, and responsibilities has yet to be reached by all concerned. The idea of a medical home to provide a systematic reporting and follow-up scheme illustrates the need for a concerted effort between states and a national guideline or system for use in newborn screening programs.

The lack of standardization is not limited to test selection, follow-up, funding, or treatment issues. There exists a great deal of variability in the analytical methods used for screening and confirmatory testing. PKU testing was traditionally screened using a bacterial inhibition assay, known as the Guthrie test, followed by confirmatory testing.[8] While some states still use this method, others screen using automated fluorometric or chemiluminescent assays, while others use tandem mass spectrometry. These differences hold true for other newborn screening tests as well. Each method and testing environment brings with it a lack of uniform cut-off and reference ranges. Testing using similar methods doesn't mean that the cut-off values for repeat or confirmatory testing are the same between laboratories. As with any laboratory test, the ideal test is one in which a certain level would mean the infant had the congenital defect, while another level would clearly differentiate those who did not. The predictive value of any newborn screening test suffers from the same ambiguity associated with any laboratory test. A repeat test, even when due to a borderline or false positive results, causes a great deal of parental anxiety and requires parental education. An additional dimension in many inherited disorders is that changes in the measured analyte may not be due to a single defect, gene, or even a particular enzyme, further complicating the proper classification of a result. Even when DNA analysis detects a mutation, the scientific information may not prove useful towards treatment or evaluation. Interpretation of results requires specialized expertise, including at least a bachelor's degree in clinical laboratory science, and a laboratory performing a sufficient quantity of tests.[9] While no universally accepted standard exists, a threshold number of 30,000 samples annually has been suggested.[10] Incorporation of minimum national standards will address the variability currently practiced between newborn screening testing facilities and contribute towards more equitable newborn screening for the more than 400 million babies born annually in the United States and its associated territories.

SAMPLING CONSIDERATIONS

Newborn screening blood samples, taken by heel stick, fill several circles on a filter paper sampling device, forming 'blood spots'. This device serves several purposes: as the sample requisition and transport device, and as a means of storage for future testing. Blood samples should be collected as late as possible before the infant is discharged, but no later than 72 hours of age. The optimum sampling time in a full-term healthy infant is between 48 and 72 hours of age. Newborn screen samples should always be drawn before a blood transfusion and not before 24 hours of age. Discharging a baby prior to 24 hours of age, necessitates a return trip to the facility to provide a blood sample, which in itself can pose numerous problems for the newborn screening program as well as the parents. In the event the sample is drawn before 24 hours of age, a second sample should be obtained by two weeks of age, but this requirement may vary by state. Infants with low birth weight, born pre-term, treated by antibiotics, or otherwise sick, should be drawn for their newborn screening tests no later than seven days of age.

Since the site of collection and testing most often occurs in different locations, transportation of samples to the testing facility, usually by a courier service or via the postal service, is required. Delivery of samples should occur promptly and samples need to be maintained at an appropriate temperature. The blood spot samples should be kept cool since any heat damage may interfere with the test results.

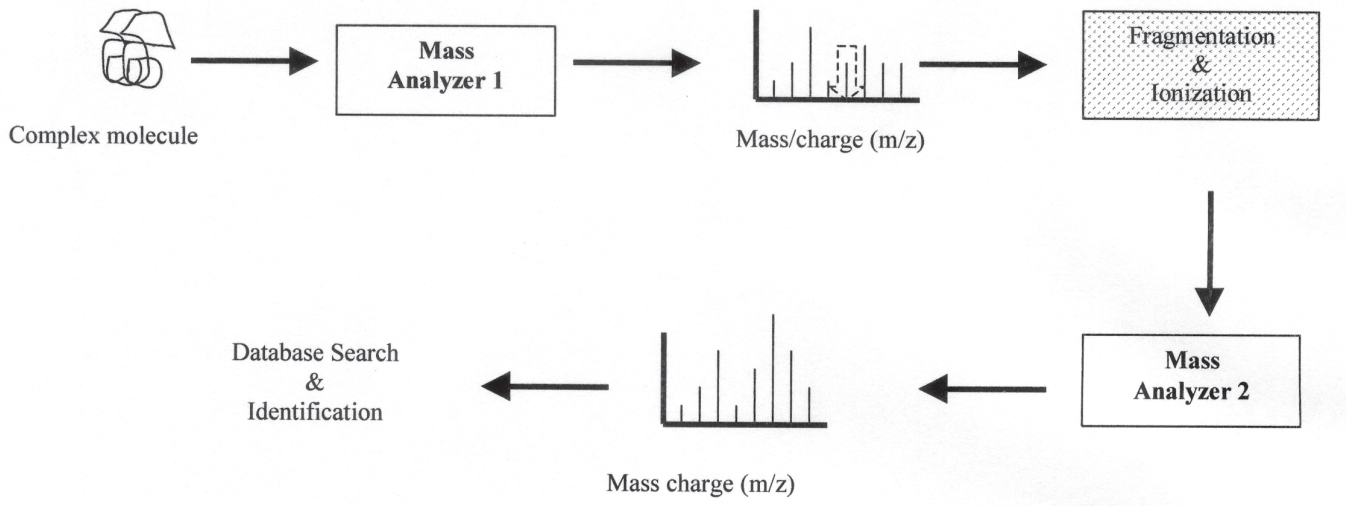
Once the newborn screening heel stick blood spots are received by the testing laboratory, they are very stable when saved under optimal conditions. Almost all infants screened have residual blood samples, even after repeat testing, and testing laboratories retain these samples. The length of time samples are saved varies between states. For example, ten programs save samples for 21 years or more, six programs for five to seven years, two programs for one to three years, six programs for six to twelve months, and five programs for one to four weeks.[11] Debate as to whether or not these residual samples should be used for later biochemical, forensic, or genetic analyses exists, but these samples remain a rich resource for future analytical, epidemiological, environmental, and clinical research. Residual samples have already been used in numerous validation studies.[12] Another use may be in forensic testing, since the heel stick blood sample may be the only verifiable biological sample for some children. Testing may be essential in postmortem identification of a genetic condition associated with the child's death. One state has decided to store newborn blood spots indefinitely to permit identification of children who are kidnap victims.[13] These ideas bring up a host of separate ethical considerations. Residual samples fall into two major classifications: identifiable (can be linked to personal information) or unlinked (anonymous). Some topics for debate include consent for research or for purposes other than newborn screening, issues of privacy and confidentiality, or even whether human biological samples should be used for research purposes at all. Another persistent ethical issue in newborn screening is whether screening is voluntary or mandatory. A voluntary program requires informed consent for testing. The potential advantage includes more prompt and efficient communication in cases requiring follow-up or for possible incorporation in experimental or pilot programs. A mandatory approach requires parents to object to and often refuse testing, without fully understanding the implications of that refusal. Only 13 states require that parents be informed before testing takes place.[14] Strategies, guidelines, and legislation governing these issues will need to be developed in the near future.

ADVANCES IN SCREENING METHODS

Over the past 40 years, technology has allowed the medical community to recognize hundreds of disorders linked to inherited metabolic defects, even in asymptomatic individuals. The expansion of molecular testing and DNA screening, along with the completion of the Human Genome project, will further provide laboratorians with the tools necessary for specific identification of existing and newly identified genetic disorders. More recently, the use of non-isotopic immunoassays and tandem mass spectrometry (MS/MS) allows fast detection of an increasing number of tests performed on a single sample. In several states, the availability of MS/MS allows more than 30 different enzyme deficiencies to be included as a part of the universal newborn screen. The number continues to grow.

Mass spectrometry, paired with gas or liquid chromatography, has been a valuable analytical tool since the mid-1960s.[15] Early on, the expense and required maintenance limited its utility in routine clinical settings. Advances in technology and streamlined models currently make this technology feasible for more facilities and further expansion of testing. The basic principles remain the same, in that a mass spectrometer measures the mass-to-charge (m/z) ratio of ionized molecules. Inherited metabolic diseases involve some abnormality of an amino acid, organic acid, fatty acid, or entire protein. These molecules are easily charged or ionized (z) either directly or through suitable derivatization. In traditional mass spectrometry, the initial step in the process requires isolation of

Figure 1. Tandem mass spectrometry (MS/MS)



the component of interest using time-consuming liquid or gas chromatography. The stream then flows through a mass analyzer and identification is made based on the fragmentation pattern. While chromatographic processes may still be coupled to MS/MS, they limit the number of compounds that can simultaneously be analyzed with a single mass analyzer and only compounds that are or can be made volatile are feasible.

MS/MS incorporates two or more mass spectrometers (the original idea behind the term 'tandem'-whether in space or time) separated by a collision chamber. Compounds are isolated using one mass analyzer according to their m/z ratio. Further fragmentation of the sample in a collision chamber (daughter ions) followed by subsequent mass analysis provides the laboratorian with an ion spectrum of specific structural information for that generation of fragment ions in a matter of minutes (Figure 1). Sequential searching of the daughter ions enables analyses of many structurally related substances from a given blood spot sample, making it an ideal newborn screening tool. Compounds in fractions of nanomoles can be separated and identified from one sample and the process, from start to finish, takes about three hours. To ease identification, samples may be derivatized, such as the interaction of blood organic acids with butanolic HCl (butylation) prior to analysis resulting in readily identifiable daughter ions. The entire process is automated.[16] The ionization process has undergone significant modifications, so that 'softer' methods using lower energies cause less fragmentation of the target molecules, making it ideal for use with amino acids, peptides, and organic acids.[17] Even when using this method, confirmation of a positive or inconclusive result remains necessary, often using DNA testing.

INHERITED METABOLIC DISORDERS

Inherited metabolic disorders, also known as in-born errors of metabolism, interrupt some aspect of a metabolic pathway changing the concentration of precursors or altering the function of physiologically important compounds, such as transport or carrier proteins. This may be due to a pathway enzyme that is either absent or one in which there is altered activity resulting in a buildup of some intermediate metabolite above the expected range and may serve as the basis for many newborn screening tests. It may be the absence of an appropriate product or insufficient substrate that is the underlying etiology of the disorder, not the abnormally high level of metabolite that is measured as a biochemical marker of the disease.

An early difficulty in diagnosing inherited metabolic disorders was associated with the fact that clinical manifestations are not always readily apparent. The most severely affected newborns may present with clinical symptoms, including unexplained failure to thrive, coma, or even death, within one week of life. However, some clinical symptoms delay until the infant's system is stressed, as with a first earache, fasting, or a change in diet, puberty, or adulthood. It should be noted that, at the time, there was little understanding of inherited diseases and the incidence of those that were recognized was considered quite rare.

Table 1. Estimated incidence in U.S. of selected inherited defects

Disorder	Incidence
Biotinidase deficiency	1:60,000-125,000
Congenital adrenal hyperplasia	1:12,000-15,000 1:300 Yupik Eskimos
Congenital hypothyroidism	1:4,000 1:2,700 Hispanic 1:700 Native Americans
Congenital toxoplasmosis	1:10,000
Cystic fibrosis	1:2,500
Fatty acid oxidation disorders*	
Medium chain	1:10,000
Long chain	1:50,000
Galactosemia	1:1:60,000-80,000
Hemoglobinopathies*	1:58,000 1:400 African Americans 1:1,100 Hispanics (Eastern States) 1:2,700 Native Americans 1:11,500 Asian Americans
Homocystinuria	1:80,000-100,000 1:760 (Mennonite population)
Hyperphenylalanemias (including PKU)	1:10,000-15,000
Maple syrup urine disease	1:200,000
Organic acidemias†	1:30,000-50,000

* Sickle cell disease
† Incidence data varies

Table 1 provides estimated incidence of selected disorders in the United States. Disorders involved in infant mortality went unrecognized as genetic defects for many years, particularly when the presenting signs and symptoms lacked a link to any known metabolic pathway deficiencies, or went unsuspected because the parents and any siblings were healthy. In addition, the signs and symptoms in metabolic diseases may appear non-specific, vary in their expression, or be attributed to more common disorders. Clinical symptoms include metabolic acidosis; hypoglycemia; cardiac, hepatic, and renal failure; cataracts; mental retardation; seizures; skin rash; Fanconi's syndrome; poor feeding; irritability; constipation and vomiting in infants; adrenal crisis (salt wasting); shock; ambiguous genitalia; developmental delays; and sudden infant death. This array of findings may not be present in all disorders.

Newborn screening testing is routinely performed in specialized private or public health laboratories, and for many scientists, the screening tests and the associated disorders often remain an academic discussion. Most curricula include discussions of phenylketonuria (PKU), galactosemia, cystic fibrosis, maple syrup urine disease, congenital hypothyroidism, and the hemoglobinopathies. More recently, additional screening for metabolic disorders include congenital adrenal hyperplasia, biotinidase deficiency, homocystinuria, fatty acid oxidation defects, organic acidemias, and a variety of amino acidemias. Universal screening (all 50 states plus the District of Columbia) exists for classical PKU and congenital hyperthyroidism. MS/MS is used in 15 states in selected populations or as part of pilot programs. North Carolina possesses the most comprehensive mandated panel to date. Table 2 provides a listing of the specific tests performed in each state as of May 2002, with an overall summary in Figure 2. The best resource for the most up-to-date offerings is the respective state's Department of Health.

SELECTED DISORDERS[18]

Phenylalanemia

PKU, an autosomal recessive disorder, is caused by either a gene mutation causing a deficiency or absence in the activity of the enzyme phenylalanine hydroxylase, an enzyme responsible for the breakdown of phenylalanine to tyrosine. This is known as classical PKU. This results in an elevation of phenylalanine but a decrease in blood tyrosine levels, both of which can be used for PKU screening. Tyrosine and its associated metabolic enzymes synthesize the important neurotransmitters, serotonin and dopamine, so a decrease has many adverse effects. Cofactor variants that also result in hyperphenylalaninemia, may cause progressive neurologic deficits, even early death. Elevated phenylalanine impairs the proper development of the central nervous system, and in untreated individuals, causes mental retardation. Convulsions, eczema, autistic-like behavior, and hyperactivity are associated findings. Since phenylalanine is an essential amino acid (obtained from the diet), treatment for classical PKU involves dietary restriction of this amino acid before the age of four weeks for the most effective treatment. The co-factor variation requires a special modified diet, low in phenylalanine but supplemented with the identified missing co-factors. Maternal PKU is problematic since high blood levels of phenylalanine are teratogenic to the fetus.

Congenital hypothyroidism

Congenital hypothyroidism results from an inadequate production of thyroid hormone due a number of different causes. In addition to the metabolic symptoms associated with hypothyroidism, patients who go undiagnosed suffer from mental retardation, neonatal jaundice, and variable degrees of growth failure, deafness, and neurologic problems. The clinical signs may not develop until the infant is several months of age or older and the most effective treatment, oral levothyroxine, should begin within the first few weeks of life. Treatment of congenital hypothyroidism has proven highly successful.

Galactosemia

Galactosemia results in elevated galactose, a monosaccharide component of the carbohydrate lactose found in cow's milk. Many commercial baby formulas contain lactose, while some of the soy-based formulas do not. The elevation of galactose is attributed to defect in the conversion of galactose to usable glucose-1-phosphate. This process occurs by several metabolic steps, and an enzyme defect in any of them can cause an elevation of galactose. Galactosemia can lead to hypoglycemia, failure to thrive, vomiting, liver disease, cataracts, and mental retardation. Symptoms can occur before the result of the newborn screen is received, but only after ingestion of galactose. At very high risk are bottle-fed babies using lactose based formulas. It is usually fatal, often associated with complications from liver failure, sepsis (*E. coli*), or bleeding, if left untreated. In treated survivors, complications include lowered IQ, which seems dependent on the time of treatment—the earlier, the better—and learning disabilities. Treated females often experience ovarian failure, or secondary amenorrhea. Complete dietary exclusion of galactose, including but not limited to lactose containing products, must be maintained for life.

Hemoglobinopathies

Hemoglobinopathies represent a group of inherited disorders due to abnormalities in hemoglobin. In newborn screening programs, states may only test for sickle cell disease, which for homozygotes, causes sickle cell anemia. In this disorder, valine is incorrectly substituted for glutamine in the sixth position of the beta chain of the hemoglobin molecule causing an increase in the red cell's fragility, particularly in low oxygen environments, resulting in hemolysis and vascular occlusions. The identification of additional hemoglobinopathies and/or thalassemias, as part of a newborn screen, varies between states.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) consists of a family of disorders arising from defects in the enzymes needed for the synthe-

Table 2. U.S. newborn screening status*

	CAH	SCD	MSUD	BIO	HCY	FOD[1]	OAD[2]	OAA[3]	MISC
Alabama	R	R	—	—	—	—	—	—	
Alaska	R	V	R	R	—	—	—	—	
Arizona	R	R	R	R	R	—	—	—	
Arkansas	—	R	—	—	—	—	—	—	
California	—	R	—	—	—	V[10]	V[8]	V[5]	
Colorado	R	R	—	R	—	—	—	—	CF
Connecticut	R	R	R	R	R	—	—	—	CF(V)
D.C.	R	R	—	R	—	—	—	G-6PD	
Delaware	R	R	R	R	—	—	—	—	
Florida	R	R	—	—	—	—	—	—	
Georgia	R	R	R	—	R	—	—	—	TYR
Hawaii	R	R	R	R	—	V[10]	V[8]	V[5]	
Idaho	—	—	R	R	—	—	—	—	
Illinois	R	R	—	R	—	—	—	—	
Indiana	R	R	R	R	R	—	—	—	
Iowa	R	R	P	P	P	MCAD(R)-P[10]	P[10]	P[7]	
Kansas	—	R	—	—	—	—	—	—	
Kentucky	—	R	—	—	—	—	—	—	
Louisiana	—	R	—	R	—	—	—	—	
Maine	R	R	R	R	R	MCAD(R)-V[5]	V[7]	V[6]	
Maryland[4]	V	V	V	V	V	—	—	—	TYR
Massachusetts	R	R	R	R	R	MCAD(R)-V[6]	V[7]	V[6]	TOX, CF(P)
Michigan	R	R	R	R	—	—	—	—	
Minnesota	R	R	P	—	P	P[9]	P[6]	P[4]	
Mississippi	R	R	—	—	—	—	—	—	
Missouri	R	R	—	—	—	—	—	—	
Montana	V	P	—	V	—	V[7]	V[7]	—	CF(V)
Nebraska	—	R	V	R	V	V[8]	V[10]	V[8]	
Nevada	—	R	R	R	—	—	—	—	
New Hampshire	—	V	R	—	R	—	—	—	TOX
New Jersey[5]	R	R	R	R	—	R[4]	—	R[2 (ASA,CIT)]	TYR
New Mexico	R	R	—	R	—	—	—	—	†
New York	—	R	R	R	R	—	—	—	HIV
North Carolina	R	R	R	—	R	R[9]	R[7]	R[4(ASA,CIT,TYR I/II)]	
North Dakota	R	V	—	—	—	P[1(MCAD)]	—	—	
Ohio	—	R	R	—	R	R[1(MCAD)]	R[3]	R[2 (ASA,CIT)]	
Oklahoma	—	R	—	—	—	—	—	—	
Oregon	—	R	R	R	—	—	—	—	
Pennsylvania	R	R	R	—	—	—	—	—	CF†
Rhode Island	R	R	R	R	R	—	—	—	
South Carolina	R	R	—	—	—	R[1(MCAD)]	—	—	
South Dakota	—	V	V	—	V	V[8]	V[9]	V[8]	
Tennessee	R	R	—	—	—	—	—	—	
Texas	R	R	—	—	—	—	—	—	†
Utah	—	R	—	—	—	—	—	—	
Vermont	—	R	R	R	R	—	—	—	
Virginia	R	R	R	R	R	—	—	—	
Washington	R	R	—	—	—	—	—	—	
West Virginia	—	V	—	—	—	—	—	—	
Wisconsin	R	R	P	R	P	R[7]	R[7]	P[3]	CF
Wyoming	—	R	—	R	—	—	—	—	CF

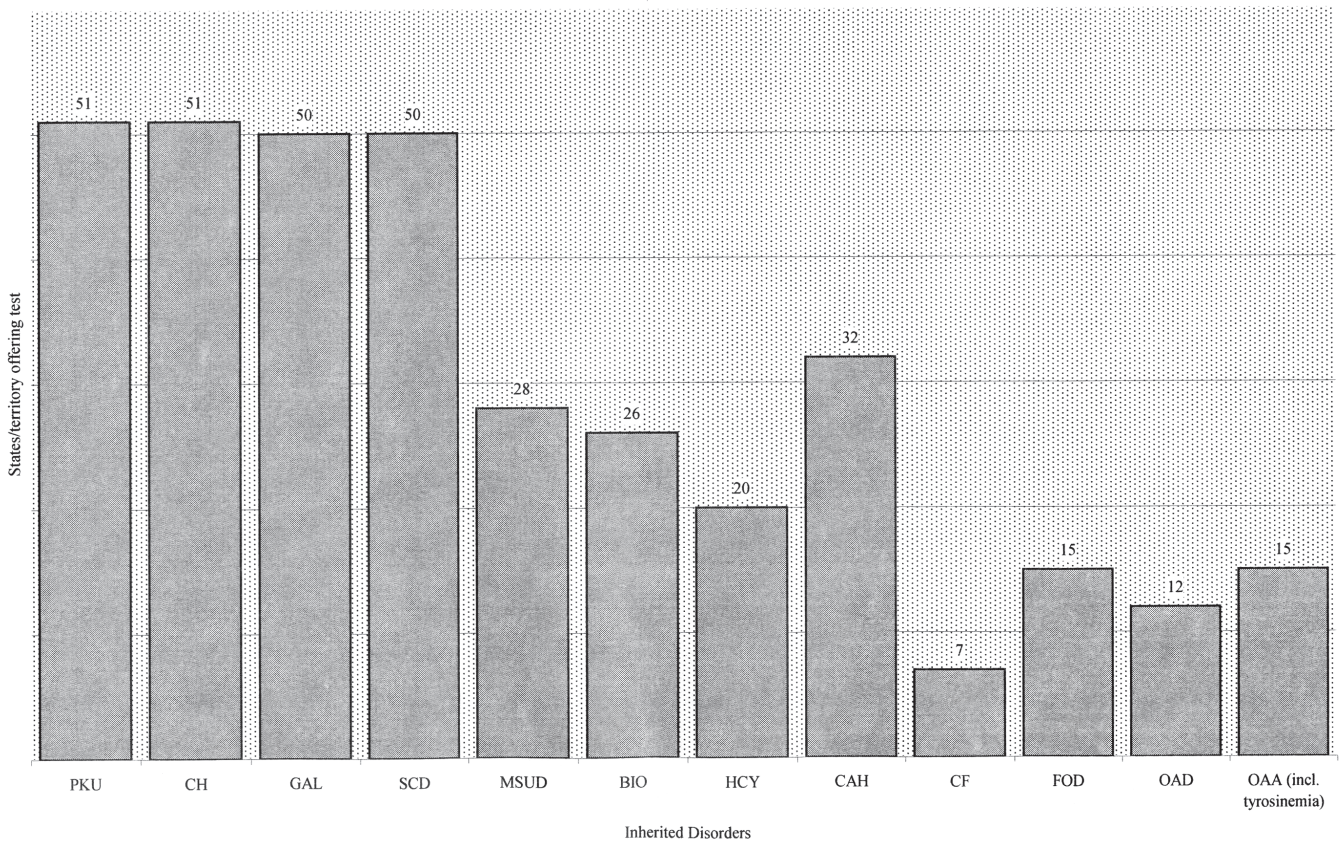
Note: All locations listed provide universal testing for PKU and congenital hypothyroidism; all except Washington perform galactosemia. These tests have not been included in this table.

* Based on composite of U.S. National Screening Status Report (NNSGRC) and Title V Block Grant FY 2000 Annual Report and FY 2002 Application, Health and Human Services Administration, Maternal and Child Health Bureau. Precise status for each area should be confirmed by contacting the local Department of Public Health.

† Supplemental testing offered; BIO = biotinidase deficiency; CAH = congenital adrenal hyperplasia; CF = cystic fibrosis; FOD = fatty acid oxidation disorders; G-6-PD = glucose-phosphate dehydrogenase deficiency; HCY = homocystinuria; MSUD = maple syrup urine disease; OAA = other amino acidemias (ASA = arginosuccinate lyase deficiency; CIT = citrullinemia); OAD = organic acidemias; P = universal pilot program; R = mandated for all newborns; SCD = sickle cell disease; TOX = congential toxoplasmosis; TYR = tyrosinemia; V = selected populations, pilot study, or as requested

1, 2, 3. Disorders listed in these are tested using MS/MS and the superscript in these categories indicate the number of disorders included.
4. State law requires that testing be offered, but since it is not mandated, considered here as voluntary.
5. Mandated by law but not yet funded.

Figure 2.



KEY: BIO = biotinidase deficiency; CAH = congenital adrenal hyperplasia; CF = cystic fibrosis; CH = congenital hypothyroidism; FOD = fatty acid oxidation disorders which include MCAD; GAL = galactosemia; HCY = homocystinuria; MSUD = maple syrup urine disease; OAA = other amino acidemias including tyrosinemia types I and II; OAD = organic acidemias; PKU = phenylalaninemia; SCD = sickle cell disease

sis of adrenal corticosteroid hormones.[19] The biosynthesis of adrenal corticosteroids begins with adrenal corticotropic hormone (ACTH) that acts on cholesterol, giving rise to pregnenolone. Pregnenolone, through the action of different enzymatic pathways, produces the mineralocorticoids (aldosterone), glucocorticoids (cortisol), androgens (testosterone), and the estrogens. The clinical consequences of the deficiency arise from the: 1) overproduction and accumulation of precursors prior to the specific blocked enzymatic step, and 2) magnitude of the hormone deficiency. Almost 90% of all cases involve a defect in 21-hydroxylase (21-OH), an enzyme needed for proper production of the mineralocorticoids (aldosterone) and glucocorticoids (cortisol). Other enzymes commonly linked to this disorder include 17-alpha hydroxylase, required for cortisol and androgen production, and 11-beta-hydroxylase needed for proper cortisol production. Because of a 21-OH defect, progesterone precursors accumulate, with some diverted to the androgen pathway. In addition, the negative feedback cortisol normally exerts on ACTH via the pituitary is impaired, causing increased ACTH production.

In utero, female fetuses exposed to excess androgens show masculinization and ambiguous genitalia at birth while male fetuses appear normal. Lack of aldosterone causes loss of sodium (hyponatremia), accumulation of potassium (hyperkalemia) which may result in a life-threatening salt-wasting syndrome, increased renin levels associated with hypovolemic shock, and decreased cortisol producing hypoglycemia. Early treatment of infants to correct the electrolyte and glucose levels can ameliorate these life-threatening symptoms and prevent incorrect sex assignment. In the absence of salt-losing phenomena, CAH may not be diagnosed until three- to seven-years of age, particularly in boys, when accelerated bone maturation that ultimately leads to short stature is detected. In milder forms, this condition can cause hirsutism and menstrual irregularities in women. Administration of glucocorticoids inhib-

its excessive production of androgens, prevents progressive virilization, and stabilizes renin levels. Adjusting the dose during times of stress, such as surgery or infection, may be necessary. The incidence in the general population of 21-OH deficiency is found to be about 1:14,000 births but with a much greater incidence in Yupik Eskimo populations where it increases to 1:680 births.

Maple syrup urine disease

Maple syrup urine disease (MSUD) is a rare disorder (1:200,000 births) due to a defect in the mitochondrial enzyme branched-chain amino acid dehydrogenase complex. Leucine, isoleucine, and valine are branched essential amino acids that are catabolized to corresponding keto acids through the process of transamination. A branched-chain keto acid dehydrogenase enzyme complex consisting of four proteins catalyzes the oxidative decarboxylation (removal of CO_2) of the resulting keto acids. Cofactors required by this complex include Mg^{2+}, thiamine pyrophosphate, Co-enzyme A, FAD, and NAD^+. The catabolism of leucine, isoleucine, and valine after the branched chain amino acid carboxylase reaction yields acyl-CoA thioesters (acetoacetate and acetyl-CoA; propionyl-CoA; acetyl-CoA, and succinyl- CoA-respectively) that serve as important lipid and carbohydrate metabolic pathway substrates. MSUD derives its name from the characteristic odor, reminiscent of maple syrup, of the urine from these patients. There are five different variants of this disease presenting an array of clinical symptoms. The most severe form, that of classic MSUD associated with the absence of branched-chain alpha-keto acid decarboxylase, results in accumulation of keto acids and their associated amino acids causing severe acidosis in the first ten days of life which, if undetected and untreated, is usually lethal in the first month. Initial symptoms may be poor eating, lethargy, and coma. Variant forms of MSUD differ in clinical presentation, depending on the severity and cause of the enzyme deficiency, and the age at diagnosis. Treatment includes lifetime dietary restriction of branched-chain amino acids and in the thiamine deficient forms, treatment with thiamine, but neurologic symptoms, including mental retardation, may persist even after treatment.

Homocystinuria

Homocystinuria represents a group of disorders related to an enzyme defect in some aspect of the interrelated catabolism of the sulfur containing amino acids (methionine, cysteine, and homocysteine). Like other inherited disorders, the severity depends on where and to what degree in the pathway the defect occurs. Methionine, linked to intermediates derived from the breakdown of folate with vitamin B_6 and B_{12} as cofactors, forms homocysteine which is further metabolized to cysteine and α-ketobutyrate. The pathway from methionine to homocysteine is reversible and low levels of homocysteine are normally reconverted to methionine as a mechanism to conserve sulfur. Problems may arise with the enzyme responsible for catalyzing the reaction of homocysteine to cysteine or with the ability to properly utilize folate and the B vitamins. The first causes elevations of both methionine and homocysteine, while the latter only homocysteine, which is diagnostic, if the screening program evaluates both substances. In either case, elevated homocysteine levels lead to homocystinuria plus serious complications that develop with age. Side effects include ocular lens problems, skeletal abnormalities, and the more serious complications attributed to arterial or venous thrombosis that may lead to an early death. Some individuals die of thrombolytic complications within the first year of life but approximately 50% of untreated individuals die by 25 years of age. Treatment depends on the source of the defect, but some individuals respond to vitamin treatment, and those non-responsive to this treatment, require specialized diets restricting methionine but supplemented with cysteine.

Biotinidase deficiency

Biotinidase deficiency testing occurs in more than twenty U.S. territories. This enzyme liberates the vitamin, biotin, to a form usable in the many dependent carboxylase (CO_2 fixing) reactions. These include enzymes involved in fatty acid synthesis, gluconeogenesis, and leucine metabolism. As part of a multi-step process, biotin becomes linked to the active site in the carboxylase enzyme and this complex is called biocytin. Biotinidase is an enzyme that cleaves the link between the enzyme and biotin. During normal catabolism, protease enzymes break down carboxylase enzymes and reclaim the associated amino acids and biocytin for recycling. Individuals with biotinidase deficiency can't regenerate biotin from biocytin leading to multiple carboxylase deficiency.[20] Affected individuals initially show combinations of neurologic and cutaneous findings, including seizures, dermatitis, alopecia, conjunctivitis, hearing loss, developmental delay, and in some instances, acute metabolic decompensation resulting in coma. Death may occur. Early treatment with biotin can prevent clinical consequences but delayed treatment may not prevent some of the less severe consequences.

Fatty acid oxidation disorders

One of the latest groups of disorders added to newborn screen menus, in a large part due to MS/MS capability, is that related to enzyme deficiencies of fatty acid oxidation. Fatty acid oxidation occurs in the mitochondria, after the fatty acid is transported from the cell cytoplasm to the mitochondria via carnitine. It continues as a series of controlled processes resulting in the breakdown of fatty acids for energy, particularly during intense exercise and the fasting state, by the liver, heart, and skeletal muscles. β-oxidation occurs as an acyl-CoA (fatty acid) dehydrogenase enzyme complex and initially removes two carbon units from an activated (CoA attached) fatty acid decreasing the original fatty acid by two carbons. This shortened fatty acid subsequently goes through repeated 'cycles' until the final production of two molecules of acetyl CoA (even numbered fatty acids). Structurally, fatty acids, derived from the diet or stored triglycerides in adipose tissue, contain a polar 'head' (-COOH) attached to repeating carbon units (CH) that make up the fatty acid 'tail' of varying length. There are different acyl-CoA dehydrogenases em-

ployed in fatty acid oxidation based on the length of the fatty acid involved. They are short- chain (SCAD), medium-chain (MCAD), long-chain acyl-CoA (LCAD), long-chain-hydroxy- (LCHAD), and very-long-chain (VLCAD) acyl-CoA dehydrogenase. They all operate in the same general manner.

In addition to the acetyl-CoA produced, each cycle of β-oxidation, via FAD, and NAD^+, shuttles electrons to the electron transport system (ETS/respiratory chain) as a mechanism for more 'direct' ATP production. A defect in one of these enzymes, from activation through the complete process of fatty acid catabolism, can cause a fatty acid oxidation disorder. Other inherited enzyme defects in the transfer of electrons to the ETS can cause increased levels of organic acids, and while related to proper fatty acid catabolism, are classified as organic acidurias. Clinical symptoms of fatty acid oxidation defects reflect the enzyme deficiency involved, but may be induced by fasting. Symptoms include lethargy, hypoglycemia, metabolic acidosis, failure to thrive, coma, and even death. MCAD, with the highest incidence found in the Caucasian population of northern European descent, may lead to death in 20% to 25% of those affected. It is estimated that 1 in 100 sudden infant death syndrome deaths is probably the result of MCAD.[21] Here again, data gleaned from newly instituted screening, whether mandated or as part of a pilot program, are sorely needed and begs the ethical issues raised earlier. Treatment includes avoidance of fasting and medium-chain fatty acids. Supplemental carnitine may also prove useful.

CONCLUSION

The selected issues and disorders discussed as part of this review serve only to highlight the many facets of newborn screening. Some states already include tests for defects such as cystic fibrosis, tyrosinemia, and glucose-6-phosphate dehydrogenase deficiency, plus many of the amino acid and organic acidurias not specifically addressed here. Advances in newborn screening, including identification of the exact genetic mutation, make the possibility of including the biochemical markers for many additional inherited defects very feasible. The ethical issues involved and the lack of nationally standardized models for many aspects of this field prove challenging. These questions will only become more complex as testing methods continue to improve.

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