IN BRIEF

Updated Neonatal Metabolic Screen

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Inherited inborn errors of metabolism (IEMs) are a relatively rare group of disorders whose identified number has grown steadily to approximately 1,000 individual disorders. This represents an aggregate incidence of 1:600 to 1:1,000 newborn infants. In the United States there are approximately 4 million births annually, translating to the potential detection of 6,000 affected newborns each year. Most affected infants are healthy appearing and asymptomatic at birth. Onset of illness ranges from hours after birth to weeks or even months of age. Early recognition and timely treatment can avert early death and long-term morbidity, emphasizing the importance of newborn screening (NBS).

In 1963, Dr Robert Guthrie developed the first NBS blood test that detected the autosomal recessively inherited disorder phenylketonuria (PKU). Guthrie's bacterial inhibition assay required a spot of blood placed on a filter paper disc transferred to an agar plate containing a substance that inhibited the growth of bacteria. Elevated levels of phenylalanine reverse the bacterial inhibition, allowing bacterial growth proportional to the level of phenylalanine in the sample of blood. Guthrie's assay was simple, was inexpensive, and, more importantly, allowed rapid and large-scale testing to be performed. PKU became a treatable disease with dramatically improved outcomes, demonstrating what expeditious detection and early treatment can accomplish. With the introduction of this method, NBS was well on its way to providing early and cost-effective diagnosis of treatable diseases.

In the 1980s, bacterial inhibition was replaced by fluorometric analysis, radioimmunoassay, and enzyme activity assays. With these techniques, expansion of the NBS nonetheless remained slow because each additional disorder required a separate test, allowing reporting on only a limited number of disorders. The 1990s brought a pivotal change in the capability of NBS with the introduction of tandem mass spectroscopy. This first-tier method expanded testing to more than 30 disorders using a single dried blood spot. This expansion was cost-effective and time-saving, allowing innumerable additions to NBS panels over time. As a consequence, concerns have arisen regarding the addition of new disorders to screening panels, especially when knowledge of disease natural history is limited. It became apparent that a consistent methodical approach was needed to standardize and inform the NBS process. The set of criteria for enrolling any disorder in population-based screening is based on a 1968 report by Wilson and Junger commissioned by the World Health Organization and has been applied to NBS: the condition 1) presents a significant population-based, well-understood health problem; 2) can be promptly identified; and 3) has early interventions available that prevent morbidity and mortality and potentially improve long-term

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outcomes. In addition, screening should be cost-effective and possess excellent sensitivity and specificity.

To address inconsistency by different NBS programs in meeting these criteria, the American College of Medical Genetics in collaboration with the US Department of Health and Human Services established a panel of disorders for mass screening referred to as the Recommended Uniform Screening Panel. Presently, the panel includes 35 primary core and 26 secondary disorders (https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html). Each state-run NBS program has jurisdiction over its selection process, creating regional variability in disorders included in screening beyond the core Recommended Uniform Screening Panel. Selecting secondary disorders for screening panels can be controversial when knowledge about the natural history of a condition is limited or when advocacy groups and lawmakers pressure states to expand screening for a disorder that does not have a treatment with proven effectiveness. For example, New York State added Krabbe disease to its panel despite the Health Resources and Services Administration's recommendation against inclusion because of the poor positive predictive value of the screening test and the uncertainty that available early intervention could reliably improve clinical outcome. Because of state-to-state variability, health-care providers should be cognizant of the disorders screened for in their respective states (https://newbornscreening.hrsa.gov/your-state). The argument can be made that the addition of a disorder to the NBS should be designed as a research question requiring parental consent.

Emerging technologies that could result in unprecedented future expansion of NBS include next-generation sequencing (NGS) gene panels and metabolomics analysis. NGS, a powerful tool that has transformed genomic medicine, has the ability to sequence the entire human genome. It includes whole genome sequencing (WGS), whole exome sequencing (WES), and targeted gene analysis of suspected disorders based on family history or phenotypic presentation.

WGS involves sequencing the entire genome, and WES involves sequencing specific coding regions of all known genes. Each of these NGS methods has its distinct technical problems. WES involves capturing specific regions of DNA, leaving the possibility of not capturing a relevant gene and ultimately yielding a false-negative test result. In contrast, WGS is not limited to a specific region, thus excluding the possibility of missing a targeted gene. However, the diploid human genome contains approximately 6.4 billion base pairs, and WGS generates massive data (approximately 200 gb), requiring an enormous amount of storage capacity. By comparison, WES analyzes smaller panels of genes and attains results significantly faster with significantly lower cost. Another significant concern with NGS is that it detects genetic variants of unknown significance (VUSs). WGS can yield 400,000 to 500,000 VUSs, and even the more limited WES detects 5,000 to 6,000 VUSs. Deciding whether any particular variant predisposes to a particular disease or signals any future clinical impact poses a real challenge.

Advocates for WGS argue that stored data can be accessed and analyzed later in a patient's life once progress has been made in identifying causative variants. In addition, public health officials can greatly benefit from access to extensive population-scale data. Although it is apparent that NGS will develop into a first-tier test, guidelines recommending NGS as a primary screening technology in NBS do not presently exist. To ensure the best use of NGS it will be prudent to implement it only for disorders that have a well-established genotype-phenotype association. Imperative is developing an algorithm that includes rapid filtering, analysis, and storage of data; timely performance of appropriate confirmatory testing; and streamlined reporting of results, all while taking into consideration the workload and financial demands placed on health-care providers and institutions.

Metabolomics, the study of the unique set of small metabolites that a particular cellular pathway produces, has considerable utility and promise in the characterization of IEMs. In humans, this encompasses identifying the complete chemical footprint of both endogenous metabolites (carbohydrates, lipids, and amino acids) and exogenous chemicals (drugs, dietary, environmental), making this analysis a reflection of both genetic and environmental factors. Metabolomics seems promising as 30% of IEMs involve defects in the metabolism of small molecules, either with substrate depleted or toxic metabolites accumulating. The Human Metabolome Database (https://www.hmdb.ca), which includes more than 100,000 metabolites, is the most comprehensive resource available for studies related to IEMs. However, as with NGS, further studies are warranted before metabolomics becomes routine in validating a candidate disorder for inclusion in NBS. For the benefits of these new technologies to be appropriately realized, any expansion should include carefully planned pilot trials on a select population.

Given that new technologies will inevitably make their way into NBS programs as they become less expensive and more efficient, the obstacles and challenges they generate will be significant, including ethical, legal, psychological, and social issues, as well as adequate funding for

appropriate infrastructure. Because NBS provides a direct benefit for the newborn infant, it does not require informed consent. In the future, however, inclusion in NBS of disorders that do not directly benefit the newborn may necessitate informed consent: conditions that do not manifest until later in childhood or even into adulthood or the identification of carrier status rather than disease.

Families are bound to experience potentially negative psychological consequences associated with the uncertainty and anxiety that results of NBS may produce. How we proceed with the implications of expanded NBS raises many important questions:

- Will NBS require pretest counseling and informed consent from parents?
- What are the ethics of reporting late-onset disorders if the disease cannot be prevented or treatment alter its course?
- Should disclosure of late-onset disorders be delayed if there is no early intervention?
- What parameters should be set for future access to and retrieval of information by public health officials?
- What financial consequences follow from disclosure of adult-onset disorders? (Although the Genetic Information Nondiscrimination Act protects patient employment rights and health insurance, it does not protect life, disability, or long-term care insurance.)

NBS has made remarkable and successful strides since Guthrie's first NBS blood test for PKU. It seems inevitable that genomic advances will progress and become incorporated into NBS as treatments for IEMs evolve. This incorporation of genomics must be carried out based on close scrutiny of benefits and harms, of long-term therapeutic effectiveness rather than as a response to public advocacy and political interests. Careful consideration must be given to its many implications: ethical, social, psychological, and legal. Expanding NBS will also place significant burdens on the health-care system, with the expanding need for educational programs, counseling resources, and follow-up programs.

COMMENTS: Without question, NBS has been an effective public health intervention over the years since Dr Guthrie's initial contribution, with testing for PKU saving thousands of lives and improving the quality of many more. But screening is only the first step. Although most states have the process of screening and then reporting well organized, what follows is most often haphazard, in effect left to chance. Especially with expanded screening, the need for a well-tailored system of follow-up becomes even more essential. So much of the benefit of screening is lost when a newborn identified with sickle cell disease does not receive early prophylactic penicillin or have access to transcranial Doppler monitoring. And from a broader perspective, the lost opportunity for us to learn from systematically gathered long-term data obviously compromises our ability to improve the quality of our care. Although costly, the investment in implementing effective systems of clinical follow-up and data collection would be repaid in its benefits to so many affected children.

–Henry M. Adam, MD
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ANSWER KEY FOR NOVEMBER PEDIATRICS IN REVIEW

Obesity in Children: 1. C; 2. E; 3. A; 4. E; 5. E.
Anxiety Disorders in Children: 1. B; 2. D; 3.B; 4. C; 5. E.
Caring for Children of Incarcerated Parents: 1. B; 2. B; 3. B; 4. B; 5. D.