Genomics in Newborn Screening

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"For in much wisdom is much vexation, and he who increases knowledge increases sorrow"

-Ecclesiastes 1:18

Newborn screening has substantially changed the genetic-metabolic world and greatly expanded the concept of preventive medicine. This expansion has been marked by two major milestones in the 50-year history of newborn screening: the first, pre-tandem mass spectrometry, included the early detection of phenylketonuria (PKU), galactosemia, homocystinuria, maple syrup urine disease, congenital hypothyroidism, congenital adrenal hyperplasia, sickle cell disease, and biotinidase deficiency; the second, tandem mass spectrometry-based, has seen an explosive increase in information, often instrumental for diagnosis, prevention, and appropriate management of many additional metabolic disorders including the organic acidemias and fatty acid oxidation defects not previously covered. The latter era, however, has also had its share of shortcomings and pitfalls, much of which related to inconclusive diagnosis and incomplete knowledge of natural history.[1-3] Determining the precise disorder in the identified infant is critical to his/her proper clinical care and treatment as well as to providing accurate information and genetic counseling to the family.

There are several possibilities for making a definitive diagnosis. In some diseases, such as tyrosinemia type I, there is a specific analyte, succinylacetone, which defines the disorder. In other disorders, represented most frequently by PKU, the metabolite profile is so abnormal and so characteristic that there is virtually no doubt as to the diagnosis. Many other disorders now included in newborn screening, however, require a determination of clearly reduced activity of the relevant enzyme or finding two pathogenic mutations in the gene that encodes the enzyme to unequivocally establish the diagnosis.[4] Proving reduced enzyme activity can be considered the gold standard but enzyme assays may require tissue not readily accessible or assays not widely available or that sometimes yield equivocal results. Determining the mutations (genotyping) is more widely available and easier to perform but its role has not been clearly formulated. The limited genotyping for 1 or only a very few mutations known to be frequent in a disorder has been implemented in the newborn screening laboratory as a second-tier test for 2 or perhaps 3 disorders. In even fewer instances, second-tier testing might allow immediate confirmation of a disorder and suggest its likely clinical effect before the identified infant receives medical evaluation.[4] Even among these few disorders, however, second-tier testing covers only the most frequent mutation(s), so evaluating the identified infant may require further examination of the gene of interest.

Although genotyping in newborn screening is currently confined to confirming the disorder suggested by the newborn screen, serious consideration is being given to examining the potential application of genomic sequencing to newborn screening itself. This consideration has emerged because of a new technology referred to as next-generation sequencing (NGS) whereby the entire sequence or a significant portion of the sequence of DNA in a newborn screening specimen could be determined. NGS could dramatically increase the number of disorders identified by newborn screening as well as identify genetic variations that indicate risk of the infant (and, by extension, family members) for subsequent development of many more disorders. Sequencing of this nature could also enhance the reliability of the confirmatory process. Although these could be great advantages, NGS in newborn screening raises many questions and very serious potential problems.

In this review we will discuss the current status of genomics in newborn screening, including the new DNA sequencing technology, its applications and limitations, as well as the inevitable clinical, ethical, and psychosocial challenges it poses when applied to newborn screening.

Current Newborn Screening

Until 2006, newborn screening varied widely among states, thus depriving many families of its benefits. Concern about this inequality led the American College of Medical Genetics (ACMG), with a commission from the Maternal and Child Health Bureau of Health Resources and Services Administration, to recommend a uniform panel of conditions for inclusion in state newborn screening programs. Although the newborn screening panel is selected by the state rather than nationally, the recommended uniform panel is now

ACMG	American College of Medical Genetics
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
PKU	Phenylketonuria
VLCAD	Very long chain acyl-CoA dehydrogenase
VUS	Variants of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

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employed throughout the US. It includes 29 mandatory conditions and an additional 25 conditions, which are part of the differential diagnosis of a condition in the core panel, or represent incidental findings for which there is potential clinical significance.[5] These conditions are listed in the ACMG website[4] and described elsewhere.[5,6]

Problems of Follow-Up in Newborn Screening

Some children identified in newborn screening do not have a defined diagnosis even after confirmatory testing with standard metabolic assays. In the infant with a fatty acid oxidation disorder, the acylcarnitine profile, which was abnormal in the newborn screening specimen may have normalized or become equivocal when the infant was clinically evaluated. In addition, overlap may occur in the metabolic profiles of several disorders delaying establishment of the correct diagnosis. This uncertainty can have a significant negative impact on the family.[7,8] In addition, screening programs aim at identifying all affected infants (ie, avoiding missing an infant, a false negative result) while tolerating only an acceptable number of false positive results.[9] This tension is exacerbated by the desire to identify not only infants with the potential for becoming severely affected but even those mildly affected whose newborn screening findings often overlap those of unaffected infants.[2,3,10,11]

An example is very long chain acyl-CoA dehydrogenase (VLCAD) deficiency, a fatty acid oxidation disorder relatively frequent among the disorders identified by expanded newborn screening, which has a severe phenotype characterized by cardiomyopathy, skeletal myopathy, liver disease, and the possibility of sudden death,[12] but also has a very mild, possibly benign phenotype.[13,14] Because the majority of newborns identified with VLCAD deficiency have remained asymptomatic through their childhood years, it is likely that they have this mild form. However, it can be difficult to differentiate the occasional neonate with the potentially severe phenotype from the neonate who may remain asymptomatic throughout life.[14] This difficulty applies to the spectrum of all disorders detected by newborn screening, not only those metabolic but also to others such as congenital hypothyroidism[15] and cystic fibrosis.[16]

To be as diagnostically precise as possible and develop some information about likely prognosis, several methods are used for medical evaluation of the infant's screening finding. These include biochemical analyses, enzyme activity measurements,[13,14,17] and genotyping. When an enzyme assay is not available or is very difficult to obtain, genotyping is critical for defining the metabolic disorder.

Genotyping for Follow-Up of Newborn Screening

Genotyping Methodologies

Following are brief descriptions of the genotyping methodologies currently or potentially used in relation to newborn screening. These methodologies are summarized in the Table. Detailed descriptions of the techniques are beyond the scope of this article.

Targeted Genotyping in Second-Tier Newborn Screening and Family Studies. When an analyte abnormality is detected in newborn screening, a frequent mutation known to be associated with the disorder may be sought by targeted mutation analysis in the newborn screening laboratory using the newborn screening specimen. This is known as second-tier testing and is most often employed to follow an abnormal initial screening result for cystic fibrosis, galactosemia, and medium chain acyl-CoA dehydrogenase deficiency. The method of choice is polymerase chain reaction (PCR) amplification of the portion of the infant's respective gene (exon) in which a known mutation resides and then applying a specific mutation detection method or hybridization technique (allele-specific oligonucleotide hybridization) to detect the mutation in the infant. The purpose of the second-tier test is to reduce the frequency of false positive results as well as provide the newborn screening program and the clinician with a sense of the urgency for clinical evaluation when urgency is required. This methodology is also employed for a family study when the proband's genotype is known.

Sanger Sequencing of Exons and Exon-Flanking Regions of a Gene. When the infant identified by newborn screening is evaluated, confirmatory testing in DNA obtained from a venous blood specimen may include sequencing the gene of interest or a critical portion of the gene. This involves PCR amplification of all exons and exon-flanking regions of the gene associated with the suspected disease and

Table. Genomic technologies used in relation to newborn screening or confirmation

Method	ASO hybridization	Sanger sequencing	NGS
Principle	• Targeted; detects one particular mutation	• Sequences gene to find mutations	• Sequences large gene panels, all exons, or entire genome to find mutations and other variations
Application	• Second-tier newborn screening • Family study	• Confirmation • Finds new mutation	• Confirmation • Finds new mutation
Turnaround	• Days	• Weeks	• Weeks-months
Cost	• Inexpensive	• Moderate to expensive • Proportional to size and number of genes sequenced	• Moderate to expensive • Lowest price per number of positions sequenced
Availability	• Available	• Available	• Limited

ASO, allele-specific oligonucleotide.