Newborn screening for genetic disorders: Current status and prospects for the future

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ABSTRACT
Newborn screening (NBS) is a public health service aimed at identifying infants with severe genetic disorders, thus providing effective treatment early enough to prevent or ameliorate the onset of symptoms. Current NBS uses biochemical analysis of dried blood spots, predominately with time-resolved fluorescence immunoassay and tandem mass spectrometry, which produces some false positives and false negatives. The application of enzymatic activity-based testing technology provides a reliable screening method for some disorders. Genetic testing is now commonly used for secondary or confirmatory testing after a positive result in some NBS programs. Recently, next-generation sequencing (NGS) has emerged as a robust tool that enables large panels of genes to be scanned together rapidly. Rapid advances in NGS emphasize the potential for genomic sequencing to improve NBS programs. However, some challenges still remain and require solution before this is applied for population screening.

KEYWORDS
Genetic disorders, Genetic testing, Newborn screening, Next-generation sequencing, Tandem mass spectrometry

INTRODUCTION
Newborn screening (NBS) is the process by which infants are tested for genetic disorders, most of which are considered to be severe, with significant morbidity and mortality. The purpose of NBS programs is to detect infants before symptoms manifest. Treatment is available, so intervention can be provided early enough to reduce the potential disabilities or death, allowing affected children to live healthier lives. The history of NBS began with phenylketonuria (PKU) screening in the early 1960s, which achieved much success. However, a new question has been raised subsequently: Which disorders should be detected? In 1968, commissioned by the World Health Organization, Wilson and Jungner developed criteria to assess the value and appropriateness of NBS programs, some of which were modified. With the Wilson and Jungner criteria as a guideline, new disorders were gradually added into the screening panels, which vary greatly between regions, mainly depending on local prevalence. However, dominated by single-disease screening, NBS expanded slowly and failed to meet the needs of population screening.
due to obvious limitations including low efficiency and high false positive/negative results. A revolutionary turning point came in the 1990s with the introduction of tandem mass spectrometry (MS/MS), which makes it possible to screen for a large number of inherited metabolic disorders (IMDs) simultaneously in a single assay, improving the efficiency of NBS enormously and expanding the NBS panel. Enzymatic activity-based testing technology also provides a reliable screening method for some disorders. Recently, with advances in next-generation sequencing (NGS), genomic testing technologies have emerged as powerful tools to identify more disorders, especially serious conditions that cannot be diagnosed with traditional tests, presenting potential applications for NBS in the foreseeable future. However, numerous challenges still remain and need to be addressed. In addition, the development of new and improved therapeutics has also led to inclusion of new diseases in NBS programs.

**SPECIFIC METABOLIC TESTING-BASED NBS**

**Single-disease screening**

In 1963, Professor Guthrie et al. reported a bacterial inhibition assay for the detection of phenylalanine to screen for PKU, pioneering the history of NBS. Later, the inclusion of congenital hypothyroidism (CH) in NBS programs was made possible by the development of radioimmunoassay in 1974. Guided by the Wilson and Jungner criteria, NBS programs gradually add other certain disorders into panels, which differ among regions. One of the most probable reasons might be associated with the local prevalence of particular disorders. However, attempts to expand the panels seemed to be difficult. They were based on the paradigm of detecting one disorder in one assay. Few disorders were added into NBS programs until the 1990s, and they most commonly included galactosemia, congenital adrenal cortical hyperplasia, glucose-6-phosphate dehydrogenase deficiency, maple syrup urine disease, homocystinuria, and cystic fibrosis. Among them, screening for PKU and CH, with high prevalence, was commenced in many countries, resulting in greatly improved outcomes. In recent years, scientific advances have led to a better understanding of genetic disorders and the availability of improved therapeutics, allowing the inclusion of some new diseases in NBS panels. For example, the fast approval of drugs related to Duchenne muscular dystrophy (DMD), subsequent with more available options for the treatments, allowed many NBS programs to provide new assays to screen for DMD, a lethal progressive X-linked mode of inheritance affecting approximately 1:5000 live male births worldwide. Historically, the primary approach to DMD NBS has been through the detection of creatine kinase enzyme activity by fluorescence measurement, which led to a higher number of false positive results. In 2021, Bao et al. established a NBS system for DMD through assessment of the MM isoenzyme of creatine kinase (CK-MM) activity by time-resolved fluoroimmunoassay, with 10 252 male neonates screened. Genetic testing was carried out for four cases whose CK-MM level was greater than 700 ng/mL and ultimately two cases were diagnosed, an incidence of 1 in 5216. As a result, the feasibility of this method of NBS for DMD was demonstrated. Although single-disease screening brings benefits, the process is slow and laborious in which one metabolite is analyzed in one test for one disorder, showing obvious limitations of low efficiency as well as a large number of false positives/negatives. Given that screening results could be easily affected by some factors such as gestational age, birthweight, time of blood collection, and selection of positive cutoffs, a second screening test is usually needed.

**Multidisease screening**

In the 1990s, the advent of tandem mass spectrometry (MS/MS) promoted the rapid development of NBS. Compared with traditional screening technologies, MS/MS allows for simultaneous detection of several diseases in one dried blood spot (DBS), greatly improving screening efficiency. Tandem mass spectrometry has emerged as an effective method in NBS, which currently enables upwards of 50 IMDs to be identified in one single analysis, including amino acids disorders, organic acidurias, and mitochondrial fatty-acid oxidation. For example, Zhao et al. reported the NBS outcomes of three million newborns who underwent MS/MS screening for IMDs in the NBS center of Zhejiang province from 2009 to 2018. Twenty-eight diseases were diagnosed and the overall incidence was 1 in 4187. This study also indicated that incremental cost–utility ratio for the screened group was CNY $768 428.76/quality-adjusted life year compared to the nonscreened group and the benefit-cost ratio was 6.09, which demonstrated that NBS using MS/MS could be considered cost effective. Nowadays, the MS/MS method has been expanded to screen for additional disorders, such as congenital adrenal cortical hyperplasia, severe combined immunodeficiency (SCID), lysosomal storage disease (LSD), and sickle cell disease. Although MS/MS is a milestone in NBS, some bottlenecks remain: (1) Screening results are susceptible to false positive results due to factors including gestational age, birthweight, nutrition, regional or ethnic differences, and medication, resulting in maternal and family anxiety; (2) High numbers of false negative results occur for some disorders such as citrin deficiency, multiple acyl-CoA dehydrogenase deficiency, methylmalonic aciduria and maple syrup urine disease in which
metabolites are usually normal during the neonatal period or in the absence of disease, leading to misdiagnosis.\(^{(20)}\) (3) It is a metabolite test and cannot determine genotype; (4) There is another group of common disorders that are not covered by MS/MS, such as osteogenesis imperfecta, Wilson’s disease, and disorders of glucose metabolism.\(^{(21,22)}\)

**ENZYMATIC ACTIVITY TESTING-BASED NBS FOR LSDs**

Based on the availability of enzyme replacement therapies, LSDs are candidates for NBS. Chamoles and co-workers were the first to develop the fluorometric method for analysis of lysosomal enzyme activities in DBS.\(^{(23–26)}\) This method has been adopted by most NBS laboratories because of easy storage and transportation, as well as high sensitivity. For example, Kang et al. investigated a large NBS program for Gaucher disease by fluorometric assay. A total of 80 855 newborns were screened and three had positive biochemical results, of which one was confirmed to have Gaucher disease by genetic testing.\(^{(27)}\) The validity of the fluorometric assay is validated; however, it is impossible to measure the activity of various enzymes simultaneously. Recently, MS/MS have been applied in LSD screening programs in many countries, with the advantage of high specificity and high sensitivity, and the ability to measure multilysosomal enzymatic activities simultaneously, which appears to be suitable for carrying out high-throughput NBS for LSDs.\(^{(28–30)}\) In 2019, Wasserstein et al. reported the New York pilot NBS program for LSD. A total of 65 605 newborns were screened and 23 were diagnosed, including one infant with Pompe disease, 15 infants with Gaucher disease and seven infants with Fabry disease.\(^{(31)}\) In 2020, Chein et al. reported the first 70 000 newborns screened by an 8-plex LSD MS/MS assay that included screening for mucopolysaccharidosis (MPS) I, II, 3B, 4A and 6, plus Pompe, Fabry and Gaucher diseases, emphasizing the advantage of the MS/MS method for LSD NBS.\(^{(16)}\) It has also been suggested that MS/MS assay is validated as a more specific, powerful, and efficient tool than the fluorometric assay, providing a multiplex solution of NBS for LSDs.\(^{(32,33)}\) Currently, although measurement of enzymatic activities is considered to be the first-tier method for LSDs screening, either by MS/MS or fluorometric assay, it has shown a significant number of false positive results, mainly because of the presence of pseudo deficiencies.\(^{(34)}\) Thus, genetic testing is often conducted in many NBS programs to verify the patients with low enzyme activity. In addition, recent data have shown that a second-tier analysis of biomarkers appears to be a powerful tool to reduce high false positive rates associated with pseudo deficiencies in NBS of Krabbe disease, MPS I, and MPS II.\(^{(35–37)}\)

**GENETIC TESTING-BASED NBS**

In recent years, rapid advances in genetic testing have led to its increasing use in NBS. Genetic testing could provide specific diagnosis at the molecular level or detect disorders effectively that could not be identified by current biochemical or physical assays, thus providing a basis for genetic counseling and eugenics. Generally, technologies such as Sanger sequencing, quantitative polymerase chain reaction (qPCR), and high-resolution melting analysis can be used for diseases with typical and characteristic clinical phenotypes, single-gene pathogenicity, or clear pathogenicity sites. Next-generation sequencing, a high-throughput sequencing technology, which includes panel sequencing, whole-exome sequencing (WES), and whole-genome sequencing (WGS) can be used for diseases with atypical clinical presentations, complex phenotypes, multigene pathogenicity or unclear pathogenicity sites. Multiplex ligation-dependent probe amplification also plays an important role in genetic disorders characterized by copy-number variation, such as congenital adrenal hyperplasia, DMD, and DiGeorge syndrome.

**Single-disease genetic screening**

**Genetic screening for hearing loss (HL)**

Approximately 70% of individuals with HL have a genetic etiology.\(^{(38,39)}\) Newborn hearing screening has been widely used in clinical practice. However, it is difficult to identify neonates with late-onset and progressive hearing impairment or susceptibility to ototoxic drugs. Morton and Nance were the first to propose that the genetic screening of a small number of HL-associated genes (\(GJB2, SLC26A4, \text{ and } MT-RNR1\)) could improve the detection of late-onset prelingual HL.\(^{(38)}\) Afterwards, genetic hearing screening programs have been conducted in many regions, achieving a tremendously success worldwide. Hao et al. reported a large-scale newborn deafness genetic screening of 142 417 neonates in Wuhan, China. The variants in \(GJB2, SLC26A4\) and \(MT-RNR1\) genes were assayed using qPCR. In total, 4289 (3.01%) newborns were found to carry at least one variant, suggesting that genetic screening can improve the detection rate of HL.\(^{(40)}\)

**Genetic screening for spinal muscular atrophy (SMA)**

Spinal muscular atrophy is a common autosomal recessive disorder in humans, caused by the homozygous absence of the survival motor neuron gene 1, with an incidence of 1/10 000. The approval of SPINRAZA (nusinersen), an antisense oligonucleotide drug, allowed SMA to be included in the Recommended Uniform Screening Panel (RUSP) in 2018.\(^{(41,42)}\) Unlike conventional NBS practices, SMA does not have a specific biochemical analyte; thus
NGS methods can include panel sequencing, WES and WGS. Currently, NGS is used as a second-tier test in some NBS studies, which means that children with positive biochemical results or clinical suspicion are sequenced by NGS to clarify diagnosis, guide care, and assess prognosis. Yang et al. reported a NBS program used with 536 008 newborns to screen for IMDs using MS/MS combined with NGS as second-tier testing in Jiangsu, China. A total of 194 cases were finally diagnosed with an IMD among 1033 primary screening positive cases, with 23 types of IMDs identified. This study shows that NGS can make up for the deficiency of MS/MS and reduce the false positives effectively.

Recently, technological progress and the dramatic reduction in cost have led to the introduction of NGS in some NBS laboratories as a first-tier testing. With relatively low cost, strong pertinence, wide coverage, and short turnaround time, panel sequencing seems to be a powerful tool, although it cannot be used to find some new genes or special mutations. Campen et al. designed a targeted panel to cover all coding regions of the following genes associated with disorders screened for in the United Kingdom: ACADM (medium chain acyl-CoA dehydrogenase deficiency), PAH (PKU), TSHR (CH), CFTR (cystic fibrosis), and HBB (sickle cell disease). The sensitivity was 100% and the specificity was 99.96%. Turnaround time of a primary report was within a week and the cost was approximately £71.14/sample. This research suggested that panel sequencing is feasible and cost-effective as a first-tier NBS program, although the range of variant types related to the disorder to be screened for was limited in this research.53

Recently, Hao et al. have successfully developed a panel of 465 causative genes for 596 inherited diseases to screen 11 484 babies in eight provinces of China, estimating an average of 0.95% clinical diagnosis rate of monogenic disorders. The turnaround time of a primary report, including the sequencing period of < 7 days, was within 11 days. Luo et al. also designed a panel of 573 genes related to severe inherited disorders, and performed NGS on 1127 individuals who had undergone biochemical NBS. Four newborns were diagnosed with glucose-6-phosphate dehydrogenase deficiency biochemically and genetically while an individual who was diagnosed with free carnitine deficiency by NGS showed negative biochemical results.

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WES and WGS have also been used in some laboratories in a diagnosed setting, especially for severely ill patients, due to the clear advantage of wide coverage where a large number of variations can be detected and some new
pathogenic genes or newly discovered diseases can be identified. Selecting exactly which disorders to screen for requires careful consideration of factors such as age of onset, severity, penetrance, treatability, confirmatory testing, and opportunities for surveillance.\(^5\) For example, the results from WES of 159 newborns, including 127 healthy newborns and 32 neonatal intensive care units (NICU) newborns in the BabySeq project were reported. It revealed a risk of childhood-onset disease in 15/159 (9.4%) newborns (including 10 healthy newborns and five NICU newborns) and actionable adult-onset disease risk in 3/85 (3.5%) newborns whose parents consented to receive this information. Carrier status for recessive diseases and pharmacogenomics variants was also reported in 88% and 5% of newborns, respectively.\(^5\) Willig et al. performed a retrospective comparison of a rapid WGS method (STATseq) and standard genetic testing in a case series from NICU and pediatric intensive care units (PICU). It was suggested that 20 of 35 (57%) infants were diagnosed with a genetic disease by STATseq and three of 32 (9%) by standard genetic testing (\(P = 0.0002\)).\(^5\) The current second-generation STATseq allows a time to provisional molecular diagnosis of 26 h with >99.5% sensitivity and specificity of genotypes, which indicates that STATseq appears to be an appropriate strategy as a first-tier test for infants in NICU and PICU.\(^6\) These results suggest that NGS can detect risk and carrier status effectively, providing basis for genetic counseling.

However, advantages seem to be lost as the gene panel becomes larger. Although the challenges of higher cost and greater time requirements might be addressed in the near future, other challenges still remain.\(^6\) One of the most formidable challenges is the interpretation of variants of uncertain significance (VUS). It is difficult to infer the pathogenicity of genetic variants, especially some rare or novel variants, which may often occur in general population screening. Thus, some variants are reported as VUS because they cannot be classified as either pathogenic or benign, which can cause concern for families.\(^6\) An additional obstacle is the frequent occurrence of unsolicited findings – unexpected findings that are unrelated to the initial reason for testing when applying genomic sequencing.\(^6\) Although criteria have been raised to help avoid unsolicited findings, they cannot be excluded entirely and controversy has arisen over which data should be reported.\(^6\) Furthermore, storage of a substantial amount of genetic data has also raised many questions, mainly including what should be stored, the high cost of storing and stewarding these data, and the potential risk of disclosure and breaches of privacy.\(^6\)

Despite the increasingly attractive usage of NGS, its implementation as a general practice is therefore still premature with substantial challenges to be addressed, and it is unlikely to – and should not – replace present screening methods.\(^6\)

**SUMMARY**

In conclusion, NBS has evolved as a standard component of preventive public health and its panel is continuously expanding. Specific metabolic testing technologies based on DBS still play an important role in NBS. With scientific advances, genetic testing and genomic testing have emerged as a powerful tool. However, both have their advantages and disadvantages. For some genetic disorders, the integrated analysis of metabolic and genetic data will improve the current NBS efficiency, enabling more affected patients to receive early diagnosis and treatment, improving their prognosis.

**CONFLICT OF INTEREST**

None.

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