REVIEW

WILEY

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

Si Ding | Lianshu Han

Department of Pediatric Endocrinology and Genetic Metabolism, Xinhua Hospital, Shanghai Institute of Pediatric Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Correspondence
Lianshu Han, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, 1665 KongJiang Road, Shanghai 200092, China.
Email: hanlianshu@xinhuamed.com.cn

Received: 6 March 2022
Accepted: 27 July 2022

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.[1] However, a new question has been raised subsequently: Which disorders should be detected? In 1968, commissioned by the World Health Organization, Wilson and Jungner[2] 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

DOI: 10.1002/ped4.12343

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.[3] 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.[1] Later, the inclusion of congenital hypothyroidism (CH) in NBS programs was made possible by the development of radioimmunoassay in 1974.[4] 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.[5] 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,[6,7] a lethal progressive X-linked mode of inheritance affecting approximately 1:5000 live male births worldwide.[8] 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.[9] 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,[10–12] 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.[3] 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.[13] 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 adrenocortical hyperplasia, severe combined immunodeficiency (SCID), lysosomal storage disease (LSD), and sickle cell disease.[14–17] 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;[18,19] (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 LSD.[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 screen 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*, 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

NBS programs come with genetic assays for SMA screening and the feasibility has been demonstrated. Chien et al. reported a NBS program of 120 267 newborns for SMA using real-time PCR (RT-PCR) combined with droplet digital PCR (ddPCR) as second-tier testing in Taiwan, China. Seven of 15 screen-positive infants were finally confirmed as having SMA, and the incidence was 1/17 181.[43] Kariyawasam et al. used the same method to screen 103 903 newborns in Australia. Nine positive patients with SMA were successfully identified and the incidence of SMA was 1 in 11 544. In addition, 44% (4/9) patients evolved clinical symptoms within 4 weeks of life, with hypotonia and weakness initially recognized in the neck.[44] It is suggested that NBS can detect patients affected by SMA before symptom onset and enable early therapeutic intervention.

Genetic screening for SCID

Severe combined immunodeficiency is an X-linked or autosomal recessive disorder, which is the most severe type of primary immunodeficiency. It has been reported that the survival rate was at least 90% among infants who received transplants at 3.5 months of age or younger, regardless of donor type.[45] With high specificity and high sensitivity, quantification of T-cell receptor excision circles in DBS by qPCR has emerged as the main method of NBS for SCID.[46] In 2008, SCID NBS pilot program was conducted in America. Since then, NBS for SCID has been implemented in the United Kingdom, France, and China.[47–49] Ding et al. estimated the costs and benefits of NBS for SCID in Washington State, with 86 600 infants screened. They predict an additional 1.19 newborn infants with SCID detected preclinically through screening in comparison with those who would have been detected early through family history, and 0.40 deaths averted annually. The incremental cost-effectiveness ratio estimate is roughly $35 000 per life-year saved and a benefit-cost ratio of 5.31, finding that NBS for SCID helps improve survival and can be considered cost effective and cost beneficial.[50]

In addition, genetic screening for other diseases has been put into practice, such as X-linked aglobulinemia and fragile-X syndrome. Although considered to be a tremendously successful public health program, single disease genetic screening has certain limitations, especially low efficiency, because a screening test is used for detecting only a single identified disorder.

Multidisease genetic screening

In comparison with genetic testing, NGS is a massive parallel sequencing where large panels of genes can be sequenced simultaneously at a single assay, greatly improving efficiency. Depending on the sequencing coverage, 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.[51] 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.[52] 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 monogenetic disorders. The turnaround time of a primary report, including the sequencing period of < 7 days, was within 11 days.[54] 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. The carrier frequencies of mutations in common genes causing IMDs in China were also investigated. The top five genes with the highest carrier frequencies of mutations were *PAH* (1.79%), *ETFDH* (1.23%), *MMACHC* (1.15%), *SLC25A13* (0.98%), and *GCDH* (0.80%).[55]

WES and WGS have also been used in some laboratories in a diagnosed setting, especially for severely ill patients,[56] 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.[57] 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.[58] 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 ($P = 0.0002$).[59] 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.[60] 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.[61] 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.[62,63] 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.[56] 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.[64,65] 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.[62,63,66]

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.[61,67]

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.

REFERENCES

1. Guthrie R, Susi A. A Simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics.* 1963;32:338-343.
2. Wilson JMG, Jungner G. Principles and practice of screening for disease. World Health Organization (Geneva). Accessed March 1, 2022. https://apps.who.int/iris/bitstream/handle/10665/37650/WHO_PHP_34.pdf?sequence=17
3. Millington DS, Kodo N, Norwood DL, Roe CR. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. *J Inherit Metab Dis.* 1990;13:321-324. DOI: 10.1007/BF01799385
4. Irie M, Enomoto K, Enomoto H, Umeda M. Microdetermination of TSH in dried blood spot–its use in the mass-screening for congenital or juvenile primary hypothyroidism (author's transl) (in Japanese). *Nihon Naibunpi Gakkai Zasshi.* 1976;52:243-247. DOI: 10.1507/endocrine1927.52.3_243
5. Spahis JK, Bowers NR. Navigating the maze of newborn screening. *MCN Am J Matern Child Nurs.* 2006;31:190-196. DOI: 10.1097/00005721-200605000-00012
6. Lim KR, Maruyama R, Yokota T. Eteplirsen in the treatment of Duchenne muscular dystrophy. *Drug Des Devel Ther.* 2017;11:533-545. DOI: 10.2147/DDDT.S97635
7. Timonen A, Lloyd-Puryear M, Hougaard DM, Meriö L, Mäkinen P, Laitala V, et al. Duchenne Muscular Dystrophy Newborn Screening: Evaluation of a New GSP(®) Neonatal Creatine Kinase-MM Kit in a US and Danish Population. *Int J Neonatal Screen.* 2019;5:27. DOI: 10.3390/ijns5030027
8. Mendell JR, Shilling C, Leslie ND, Flanigan KM, al-Dahhak R, Gastier-Foster J, et al. Evidence-based path to newborn screening for Duchenne muscular dystrophy. *Ann Neurol.* 2012;71:304-313. DOI: 10.1002/ana.23528
9. Bao Y, Pan X, Pan J, Pan S, Zhuang D, Li H, et al. Study on newborn screening for Duchenne muscular

dystrophy and diagnostic strategy (in Chinese). *Chin J Med Genet.* 2021;38:430-434. DOI: 10.3760/cma.j.cn511374-20200415-00270
10. Lain S, Trumpff C, Grosse SD, Olivieri A, Van Vliet G. Are lower TSH cutoffs in neonatal screening for congenital hypothyroidism warranted. *Eur J Endocrinol.* 2017;177:D1-D12. DOI: 10.1530/EJE-17-0107
11. Peng G, Tang Y, Cowan TM, Zhao H, Scharfe C. Timing of newborn blood collection alters metabolic disease screening performance. *Front Pediatr*. 2020;8:623184. DOI: 10.3389/fped.2020.623184
12. Gidlöf S, Wedell A, Guthenberg C, von Döbeln U, Nordenström A. Nationwide neonatal screening for congenital adrenal hyperplasia in Sweden: a 26-year longitudinal prospective population-based study. *JAMA Pediatr.* 2014;168:567-574. DOI: 10.1001/jamapediatrics.2013.5321
13. Zhao Z, Chen C, Sun X, Zhou D, Huang X, Dong H. Newborn screening for inherited metabolic diseases using tandem mass spectrometry in China: Outcome and cost-utility analysis. *J Med Screen.* 2022;29:12-20. DOI: 10.1177/09691413211021621
14. Matern D, Tortorelli S, Oglesbee D, Gavrilov D, Rinaldo P. Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: the Mayo Clinic experience (2004-2007). *J Inherit Metab Dis.* 2007;30:585-592. DOI: 10.1007/s10545-007-0691-y
15. Azzari C, la Marca G, Resti M. Neonatal screening for severe combined immunodeficiency caused by an adenosine deaminase defect: a reliable and inexpensive method using tandem mass spectrometry. *J Allergy Clin Immunol.* 2011;127:1394-1399. DOI: 10.1016/j.jaci.2011.03.040
16. Chien YH, Lee NC, Chen PW, Yeh HY, Gelb MH, Chiu PC, et al. Newborn screening for Morquio disease and other lysosomal storage diseases: results from the 8-plex assay for 70,000 newborns. *Orphanet J Rare Dis.* 2020;15:38. DOI: 10.1186/s13023-020-1322-z
17. Lobitz S, Klein J, Brose A, Blankenstein O, Frömmel C. Newborn screening by tandem mass spectrometry confirms the high prevalence of sickle cell disease among German newborns. *Ann Hematol.* 2019;98:47-53. DOI: 10.1007/s00277-018-3477-4
18. Peng G, Tang Y, Gandotra N, Enns GM, Cowan TM, Zhao H, et al. Ethnic variability in newborn metabolic screening markers associated with false-positive outcomes. *J Inherit Metab Dis.* 2020;43:934-943. DOI: 10.1002/jimd.12236
19. Ryckman KK, Berberich SL, Shchelochkov OA, Cook DE, Murray JC. Clinical and environmental influences on metabolic biomarkers collected for newborn screening. *Clin Biochem.* 2013;46:133-138. DOI: 10.1016/j.clinbiochem.2012.09.013
20. Estrella J, Wilcken B, Carpenter K, Bhattacharya K, Tchan M, Wiley V. Expanded newborn screening in New South Wales: missed cases. *J Inherit Metab Dis.* 2014;37:881-887. DOI: 10.1007/s10545-014-9727-2
21. James PM, Levy HL. The clinical aspects of newborn screening: importance of newborn screening follow-up. *Ment Retard Dev Disabil Res Rev.* 2006;12:246-254. DOI: 10.1002/mrdd.20120
22. Lin Y, Liu Y, Zhu L, Le K, Shen Y, Yang C, et al. Combining newborn metabolic and genetic screening for neonatal intrahepatic cholestasis caused by citrin deficiency. *J Inherit Metab Dis.* 2020;43:467-477. DOI: 10.1002/jimd.12206
23. Chamoles NA, Blanco M, Gaggioli D. Diagnosis of alpha-L-iduronidase deficiency in dried blood spots on filter paper: the possibility of newborn diagnosis. *Clin Chem.* 2001;47:780-781.
24. Chamoles NA, Blanco M, Gaggioli D, Casentini C. Gaucher and Niemann-Pick diseases–enzymatic diagnosis in dried blood spots on filter paper: retrospective diagnoses in newborn-screening cards. *Clin Chim Acta.* 2002;317:191-197. DOI: 10.1016/s0009-8981(01)00798-7
25. Chamoles NA, Niizawa G, Blanco M, Gaggioli D, Casentini C. Glycogen storage disease type II: enzymatic screening in dried blood spots on filter paper. *Clin Chim Acta.* 2004;347:97-102. DOI: 10.1016/j.cccn.2004.04.009
26. Chamoles NA, Blanco M, Gaggioli D. Fabry disease: enzymatic diagnosis in dried blood spots on filter paper. *Clin Chim Acta.* 2001;308:195-196. DOI: 10.1016/s0009-8981(01)00478-8
27. Kang L, Zhan X, Gu X, Zhang H. Successful newborn screening for Gaucher disease using fluorometric assay in China. *J Hum Genet.* 2017;62:763-768. DOI: 10.1038/jhg.2017.36
28. Lin N, Huang J, Violante S, Orsini JJ, Caggana M, Hughes EE, et al. Liquid chromatography-tandem mass spectrometry assay of leukocyte acid α-Glucosidase for post-newborn screening evaluation of pompe disease. *Clin Chem.* 2017;63:842-851. DOI: 10.1373/clinchem.2016.259036
29. Chuang CK, Lin HY, Wang TJ, Huang YH, Chan MJ, Liao HC, et al. Status of newborn screening and follow up investigations for mucopolysaccharidoses I and II in Taiwan. *Orphanet J Rare Dis.* 2018;13:84. DOI: 10.1186/s13023-018-0816-4
30. Burlina AB, Polo G, Salviati L, Duro G, Zizzo C, Dardis A, et al. Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy. *J Inherit Metab Dis.* 2018;41:209-219. DOI: 10.1007/s10545-017-0098-3
31. Wasserstein MP, Caggana M, Bailey SM, Desnick RJ, Edelmann L, Estrella L, et al. The New York pilot newborn screening program for lysosomal storage diseases: Report of the First 65,000 Infants. *Genet Med.* 2019;21:631-640. DOI: 10.1038/s41436-018-0129-y
32. Liao HC, Chiang CC, Niu DM, Wang CH, Kao SM, Tsai FJ, et al. Detecting multiple lysosomal storage diseases by tandem mass spectrometry–a national newborn screening program in Taiwan. *Clin Chim Acta.* 2014;431:80-86. DOI: 10.1016/j.cca.2014.01.030
33. Gelb MH, Scott CR, Turecek F. Newborn screening for lysosomal storage diseases. *Clin Chem.* 2015;61:335-346. DOI: 10.1373/clinchem.2014.225771
34. Clarke LA, Giugliani R, Guffon N, Jones SA, Keenan HA, Munoz-Rojas MV, et al. Genotype-phenotype relationships in mucopolysaccharidosis type I (MPS I): Insights from the International MPS I Registry. *Clin Genet.* 2019;96:281-289. DOI: 10.1111/cge.13583

35. Guenzel AJ, Turgeon CT, Nickander KK, White AL, Peck DS, Pino GB, et al. The critical role of psychosine in screening, diagnosis, and monitoring of Krabbe disease. *Genet Med.* 2020;22:1108-1118. DOI: 10.1038/s41436-020-0764-y
36. Herbst ZM, Urdaneta L, Klein T, Burton BK, Basheeruddin K, Liao HC, et al. Evaluation of two methods for quantification of glycosaminoglycan biomarkers in newborn dried blood spots from patients with severe and attenuated mucopolysaccharidosis type II. *Int J Neonatal Screen.* 2022;8:9. DOI: 10.3390/ijns8010009
37. Herbst ZM, Urdaneta L, Klein T, Fuller M, Gelb MH. Evaluation of multiple methods for quantification of glycosaminoglycan biomarkers in newborn dried blood spots from patients with severe and attenuated mucopolysaccharidosis-I. *Int J Neonatal Screen.* 2020;6:69. DOI: 10.3390/ijns6030069
38. Morton CC, Nance WE. Newborn hearing screening–a silent revolution. *N Engl J Med.* 2006;354:2151-2164. DOI: 10.1056/NEJMra050700
39. Smith RJ, Bale JF Jr, White KR. Sensorineural hearing loss in children. *Lancet.* 2005;365:879-890. DOI: 10.1016/S0140-6736(05)71047-3
40. Hao Z, Fu D, Ming Y, Yang J, Huang Q, Lin W, et al. Large scale newborn deafness genetic screening of 142,417 neonates in Wuhan, China. *PLoS One.* 2018;13:e0195740. DOI: 10.1371/journal.pone.0195740
41. Fabie N, Pappas KB, Feldman GL. The current state of newborn screening in the United States. *Pediatr Clin North Am.* 2019;66:369-386. DOI: 10.1016/j.pcl.2018.12.007
42. Kraszewski JN, Kay DM, Stevens CF, Koval C, Haser B, Ortiz V, et al. Pilot study of population-based newborn screening for spinal muscular atrophy in New York state. *Genet Med.* 2018;20:608-613. DOI: 10.1038/gim.2017.152
43. Chien YH, Chiang SC, Weng WC, Lee NC, Lin CJ, Hsieh WS, et al. Presymptomatic diagnosis of spinal muscular atrophy through newborn screening. *J Pediatr.* 2017;190:124-129.e1. DOI: 10.1016/j.jpeds.2017.06.042
44. Kariyawasam D, Russell JS, Wiley V, Alexander IE, Farrar MA. The implementation of newborn screening for spinal muscular atrophy: the Australian experience. *Genet Med.* 2020;22:557-565. DOI: 10.1038/s41436-019-0673-0
45. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. *N Engl J Med.* 2014;371:434-446. DOI: 10.1056/NEJMoa1401177
46. Modell V, Knaus M, Modell F. An analysis and decision tool to measure cost benefit of newborn screening for severe combined immunodeficiency (SCID) and related T-cell lymphopenia. *Immunol Res.* 2014;60:145-152. DOI: 10.1007/s12026-014-8485-4
47. Adams SP, Rashid S, Premachandra T, Harvey K, Ifederu A, Wilson MC, et al. Screening of neonatal UK dried blood spots using a duplex TREC screening assay. *J Clin Immunol.* 2014;34:323-330. DOI: 10.1007/s10875-014-0007-6
48. Audrain M, Thomas C, Mirallie S, Bourgeois N, Sebille V, Rabetrano H, et al. Evaluation of the T-cell receptor excision circle assay performances for severe combined immunodeficiency neonatal screening on Guthrie cards in a French single centre study. *Clin Immunol.* 2014;150:137-139. DOI: 10.1016/j.clim.2013.11.012
49. Chien YH, Chiang SC, Chang KL, Yu HH, Lee WI, Tsai LP, et al. Incidence of severe combined immunodeficiency through newborn screening in a Chinese population. *J Formos Med Assoc.* 2015;114:12-16. DOI: 10.1016/j.jfma.2012.10.020
50. Ding Y, Thompson JD, Kobrynski L, Ojodu J, Zarbalian G, Grosse SD. Cost-effectiveness/cost-benefit analysis of newborn screening for severe combined immune deficiency in Washington State. *J Pediatr.* 2016;172:127-135. DOI: 10.1016/j.jpeds.2016.01.029
51. Zhang WR, Zhao ZY. Advances in genetic screening for neonatal diseases (in Chinese). *Chin J Pediatr.* 2020;58:1033-1037. DOI: 10.3760/cma.j.cn112140-20200614-00620
52. Yang Y, Wang L, Wang B, Liu S, Yu B, Wang T. Application of next-generation sequencing following tandem mass spectrometry to expand newborn screening for inborn errors of metabolism: A multicenter study. *Front Genet.* 2019;10:86. DOI: 10.3389/fgene.2019.00086
53. van Campen JC, Sollars E, Thomas RC, Bartlett CM, Milano A, Parker MD, et al. Next generation sequencing in newborn screening in the United Kingdom National Health Service. *Int J Neonatal Screen.* 2019;5:40. DOI: 10.3390/ijns5040040
54. Hao C, Guo R, Hu X, Qi Z, Guo Q, Liu X, et al. Newborn screening with targeted sequencing: a multicenter investigation and a pilot clinical study in China. *J Genet Genomics.* 2022;49:13-19. DOI: 10.1016/j.jgg.2021.08.008
55. Luo X, Sun Y, Xu F, Guo J, Li L, Lin Z, et al. A pilot study of expanded newborn screening for 573 genes related to severe inherited disorders in China: results from 1,127 newborns. *Ann Transl Med.* 2020;8:1058. DOI: 10.21037/atm-20-1147
56. van El CG, Cornel MC, Borry P, Hastings RJ, Fellmann F, Hodgson SV, et al. Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics. *Eur J Hum Genet.* 2013;21:580-584. DOI: 10.1038/ejhg.2013.46
57. Berg JS, Agrawal PB, Bailey DB Jr, Beggs AH, Brenner SE, Brower AM, et al. Newborn Sequencing in Genomic Medicine and Public Health. *Pediatrics.* 2017;139. DOI: 10.1542/peds.2016-2252
58. Ceyhan-Birsoy O, Murry JB, Machini K, Lebo MS, Yu TW, Fayer S, et al. Interpretation of genomic sequencing results in healthy and ill newborns: Results from the BabySeq Project. *Am J Hum Genet.* 2019;104:76-93. DOI: 10.1016/j.ajhg.2018.11.016
59. Willig LK, Petrikin JE, Smith LD, Saunders CJ, Thiffault I, Miller NA, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. *Lancet Respir Med.* 2015;3:377-387. DOI: 10.1016/S2213-2600(15)00139-3
60. Miller NA, Farrow EG, Gibson M, Willig LK, Twist G, Yoo B, et al. A 26-hour system of highly sensitive whole genome sequencing for emergency management of genetic diseases.

Genome Med. 2015;7:100. DOI: 10.1186/s13073-015-0221-8
61. Landau YE, Lichter-Konecki U, Levy HL. Genomics in newborn screening. *J Pediatr.* 2014;164:14-19. DOI: 10.1016/j.jpeds.2013.07.028
62. Friedman JM, Cornel MC, Goldenberg AJ, Lister KJ, Sénécal K, Vears DF. Genomic newborn screening: public health policy considerations and recommendations. *BMC Med Genomics.* 2017;10:9. DOI: 10.1186/s12920-017-0247-4
63. Friedman E. Next generation sequencing for newborn screening: are we there yet. *Genet Res (Camb).* 2015;97:e17. DOI: 10.1017/S001667231500018X
64. Knoppers BM, Avard D, Sénécal K, Zawati MH. Return of whole-genome sequencing results in paediatric research: a statement of the P3G international paediatrics platform. *Eur J Hum Genet.* 2014;22:3-5. DOI: 10.1038/ejhg.2013.176
65. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med.* 2013;15:565-574. DOI: 10.1038/gim.2013.73
66. Howard HC, Knoppers BM, Cornel MC, Wright CE, Sénécal K, Borry P. Whole-genome sequencing in newborn screening? A statement on the continued importance of targeted approaches in newborn screening programmes. *Eur J Hum Genet.* 2015;23:1593-1600. DOI: 10.1038/ejhg.2014.289
67. Reinstein E. Challenges of using next generation sequencing in newborn screening. *Genet Res (Camb).* 2015;97:e21. DOI: 10.1017/S0016672315000178

How to cite this article: Ding S, Han L. Newborn screening for genetic disorders: Current status and prospects for the future. *Pediatr Investig*. 2022;6:291–298. https://doi.org/10.1002/ped4.12343