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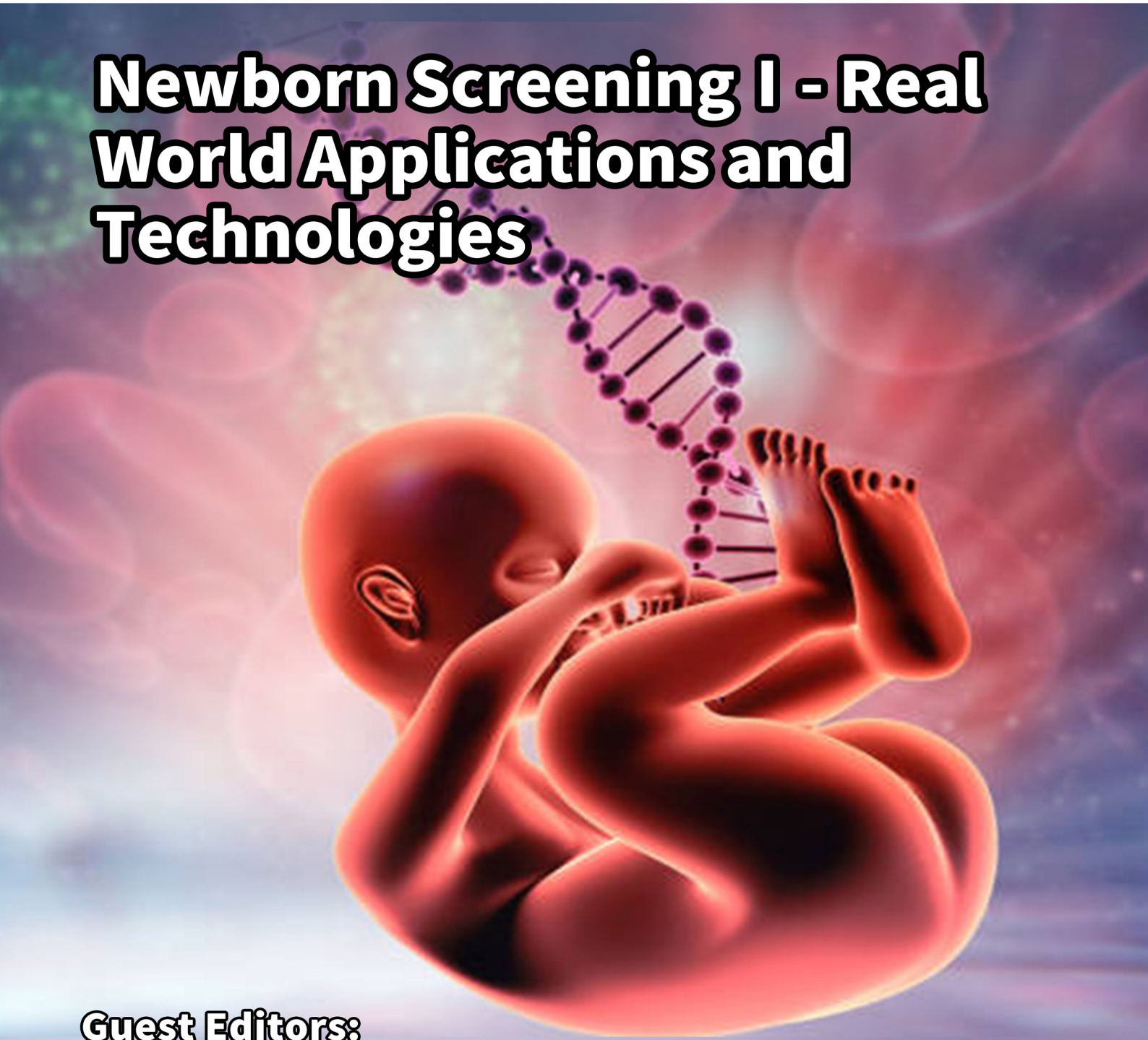
Newborn Screening I - Real World Applications and Technologies



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Virginie Bros-Facer, PhD (Illumina)
Member of Diagnostic Scientific Committee of IRDiRC

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Topic: Newborn Screening I - Real World Applications and Technologies

Prof. David A Pearce
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Research in the Pearce Lab focuses on understanding the molecular basis of several inherited pediatric neuro-degenerative diseases, including the infantile, late infantile and juvenile onset forms of Batten disease.

Dr. Pearce and his team use mouse and miniature pig models of these rare, fatal diseases to reveal molecular and cellular pathomechanisms, to identify new therapeutic targets and to test new therapeutic approaches.

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Virginie Bros-Facer received her PhD in Neurosciences from King's College London, UK followed by several postdoctoral research projects at the Institute of Neurology, UCL, London focused on testing therapeutic strategies for Amyotrophic Lateral Sclerosis. After leaving the lab, she worked for several research funding organizations in the UK including the National Institute for Health Research, the Medical Research Council and as Medical Director for Sparks, a medical research charity focusing on rare pediatric diseases. Virginie then joined EURORDIS-Rare Diseases Europe as Scientific Director where she was leading on project development and patient engagement in rare disease research projects representing the voice of rare disease patients, including within the International Rare Disease Research Consortium (IRDiRC). Just under 2 years ago, Virginie joined Illumina as Associate Director for Medical Affairs, Europe where she is engaging key opinion leaders and centers of excellence to develop clinical evidence for genetic testing of rare and undiagnosed patients to drive clinical NGS adoption and implementation in patient care. She has re-joined IRDiRC as a member of the Diagnostic Scientific Committee and coordinates a dedicated working group on real-world applications and technologies for newborn screening.

Special Issue Introduction

Newborn screening (NBS) programs are an integral part of public health systems aiming to identify infants born with childhood-onset, mostly rare disorders and initiate early intervention to improve their quality of life. Current traditional NBS programs rely on biochemical methods, and the introduction of tandem Mass Spectrometry has enabled the addition of diseases to be screened through National NBS programs. Despite these efforts, there is a significant disparity in the number of diseases screened through these programs across the world, from less than a handful in some countries to several dozens in others.

During the last two decades, technological advancements have driven the expansion of NBS pilot programs with the development of fast and accurate next-generation sequencing (NGS) technologies. NGS has opened the door to a range of possibilities in the field including, not only wide-scale implementation for confirmatory testing, but also as first-tier analysis of numerous genes associated with many genetic disorders, that can be treated presymptomatically and screened in a single test. Furthermore, with the increasing development of therapeutic strategies for rare diseases, there is an urgent need to enable the addition of diseases to be screened in a fast and efficient manner.

NGS has the potential to improve the diagnostic and prognostic utility of NBS and could enable progressive and future methodological standardization of NBS programs, leaning towards a more equitable healthcare across the world. Although true harmonization of NBS programs remain out of reach today, there is a significant potential to improve current programs so that more children and families could benefit from screening in the future.

Several pioneering initiatives in the USA, Europe, Australia, and China, are aiming to pilot NGS in NBS programs, and each initiative is designed to address specific challenges. Currently, there is not a single perfect approach which can be replicated and implemented worldwide. Each initiative has its own merit as it addresses national and/or regional needs based on piloting the technical feasibility and demonstrating clinical utility within a specific healthcare system. Concerns related to the use of these novel technologies are being addressed, including but not limited to, technical, medical, economical, ethical, and sociological aspects. These pilots do not seek perfection nor harmonization from the get-go but aim to improve the current traditional NBS programs.

The Rare Disease Research Community has a collective responsibility to aim towards a future healthcare that is more equitable and accessible. Hence, the International Rare Disease Research Consortium (IRDiRC) and an extended group of experts got together to shed light on this field, increase visibility of ongoing efforts, highlight current and future potential to expand NBS using NGS technologies and provide concrete opportunities to further the development of real-world applications for the benefit of rare disease patients and their families. Because, yes, according to Wilson and Junger as well as public health authorities the child should be the primary beneficiary of NBS, and rightly so but should you ask families who have a child with a rare disease, they will most likely tell you: it affects us all.

Dr. Virginie Bros-Facer

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GENERAL INFORMATION

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Rare Disease and Orphan Drugs Journal (*RDODJ*), is an international peer-reviewed, open access, online journal.The *Rare Disease and Orphan Drugs Journal* (*RDODJ*) is an international, peer-reviewed, open access journal for the publication of innovative research works on various aspects of this rapidly growing multidisciplinary and interdisciplinary field.

RDODJ will report on scientific advances in the genetics of rare diseases, the molecular basis of the pathologies, and translational research on diagnosis, prevention and treatment.

In addition, *RDODJ* aims to provide a forum for scientific studies and discussion covering the important regulatory, socio-economic and human science issues related to rare diseases and orphan drugs.

The ultimate objective of *RDODJ* is to promote the dissemination of research results and scientific discussion among the research community, practitioners, and patient-advocacy organizations.

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Rare Disease and Orphan Drugs Journal

Systematic Review

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A systematic review of real-world applications of genome sequencing for newborn screening

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Abstract

Aim: With the costs of genomic sequencing falling quickly and an ever-increasing number of clinical laboratories equipped with new-generation sequencing machines, healthcare systems around the world are getting ready to enter the era of genomic newborn screening (NBS). However, the adoption of Genomic Sequencing (GS), encompassing whole-exome sequencing (WES) and whole-genome sequencing (WGS), in NBS programs raises a number of clinical, ethical, and legal questions as well as organizational and economic challenges. This systematic review is part of a feasibility study to assess the introduction of WGS for NBS in Lombardy region with the specific aim of gathering evidence from existing pilots in the field whose results have been published.

Methods: Three different sources were identified for the selection of articles in order to obtain a various and unbiased set of publications. 33 articles were retained for analysis to answer the following questions:
1. Clinical: Does genomic sequencing demonstrate clinical utility in the context of NBS? What are the limitations of these kind of programs?
2. Societal: What are the social, ethical and psychological implications of using GS for NBS?
3. Governance: What are the legal, economic, and organizational challenges for GS-based NBS programs?

Results: There is a general consensus in the literature on the key principles that should guide the adoption of GS in NBS, such as the inclusion of actionable genes only, the need for informed consent from the parents, the right of the newborn to an open future, which means the exclusion of late-onset diseases even when those are considered treatable. However, there are still several differences in how these principles are detailed and applied.

Conclusion: Real-world evidence from a handful of pilot projects (namely BabySeq and NC-Nexus, both carried out

in the USA) have been published recently; however, this evidence is not yet sufficient to put an end to the broad and animated debate on the use of GS for NBS. Ethical, legal, and social issues still constitute great challenges and major barriers to wide and uniform adoption of GS in NBS. On the clinical side, a number of issues remain unaddressed, such as the benefits and limitations of the different approaches (targeted sequencing, GS only versus GS+standard NBS), the genes/diseases to include and the frequency of incidental findings, identification of carrier status, and variants of uncertain significance (VUS). Further pilots and consultations with involved stakeholders will be necessary before GS-based NBS can be accepted and systematically implemented in national healthcare programs.

Keywords: Newborn screening, genome sequencing, whole exome sequencing, whole genome sequencing

INTRODUCTION

Newborn screening (NBS) programs have been running successfully for more than 50 years since its introduction in the 1960s. In many countries, the first disorder included in screening programs was Phenylketonuria (PKU). With the advent of Tandem Mass Spectrometry (MS/MS), the number of conditions screened increased to around 50, although with great disparities among countries[1]. The introduction of MS/MS was therefore a key driver for the expansion of the number of conditions screened, with an increase in the order of 10 folds. Now, with the costs of genomic sequencing falling quickly and an ever-increasing capacity of laboratories as more and more are getting equipped with new generation sequencing instruments, a further scale-up of NBS programs is technically possible, also in the order of 10 folds (from 50 to 500 conditions)[2-6]. However, it is important to consider that one disease can be linked to one or more genes, and for each gene, there could be several variants, pathogenic or not. A major limitation of the GS approach is that several variants cannot be classified either as non-pathogenic or pathogenic and are actually classified as variants of uncertain/unknown significance (VUS). The specific criteria for selecting the genes and the conditions to be screened are not yet unanimously accepted, even if there is a general agreement that only pathogenic or likely-pathogenic variants should be reported and the principles set by Wilson and Jungner are still basically valid[7]. Moreover, the adoption of Genomic Sequencing (GS), meaning whole-exome sequencing (WES) or whole-genome sequencing (WGS), poses a number of clinical, ethical, and legal questions[8-12] together with organizational and economic challenges[3,13-14].

This systematic review is part of a feasibility study assessing the introduction of GS for NBS in Lombardy region (Italy) and is co-funded by the regional government (Regione Lombardia) and Fondazione Telethon. The study is conducted according to the Responsible Research and Innovation (RRI) principles[15-18] and is inspired by the EUNetHTA Core Model®[19,20]. RRI principles include, among others, engagement of all societal actors, gender balance both within the research teams and in the group of consulted stakeholders, ethics, and governance, with the intent to enable a positive impact of the research on society.

Considering the nine domains of EUNetHTA Core Model®, the purpose of this review is to inform the activities of the feasibility study in the following domains while addressing relevant and associated issues:

(1) Health Problem and Current Use of the Technology with a special focus on pilot projects that tested GS for NBS;

(2) Description and technical characteristics of the technology with a focus on the discussion within the scientific community on the list of genes that should (or should not) be included in the analysis;

(3) Safety with a focus on incidental findings, false negatives, and false positives;

(4) Clinical Effectiveness trying to answer the question: What is the number of newborns per year we could expect to identify as positive?

(5) Costs and economic evaluation to investigate which methods and models were used to estimate the costs of GS-based NBS by ongoing initiatives;

(6) Ethical analysis considering in particular that in the case of NBS the patient cannot make any decision by himself/herself, as all decisions are taken by the parents;

(7) Organizational aspects - again looking at recent pilots, trying to identify the major obstacle(s) to the full deployment as part of the standard of care of a GS-NBS program;

(8) Patients and Social aspects with a focus on the acceptability of GS-based screening programs by citizens and the methodology adopted by other pilot programs to consult and engage citizens;

(9) Legal aspects to first answer the question of whether a genomic screening program could be made mandatory (as it is now for the traditional Italian NBS program) or should be voluntary.

Trying to cover all the above-mentioned issues, we selected a wide search algorithm without limiting our review to a specific domain but limiting it to newborn/neonatal screening AND WGS (that includes, as a MeSH term, WES). For results and conclusions, we grouped the above-listed domains into three main areas: Clinical (covering issues 1 to 4), Societal (covering issues 6 and 8), and Governance (covering issues 5, 7, and 9).

METHODS

Search strategy

Three different sources were identified for the selection of the papers in order to obtain a various and unbiased set of articles. The sources included (1) the PubMed online database via a query performed on September 28th, 2022; (2) the Mendeley library shared within the clinicians working group; and (3) the final selection of articles that were selected for Downie *et al.*'s 2021 systematic review "Principles of Genomic Newborn Screening Programs: a systematic review[21]".

The search algorithm used in PubMed was defined according to the objective of the review, i.e., to provide the practical and theoretical background for the application of WGS or WES techniques to population-wide NBS programs. The search was performed for all study types published in English, with the full texts available using MeSH terms (whole-genome sequencing) AND (neonatal screening). These MeSH terms were selected because they include all the possible synonyms, and in the case of WGS, it includes WES as well. The query on the PubMed online database with this algorithm gave 147 articles as a result.

The Mendeley library has been populated by the multidisciplinary team working on the feasibility study mentioned in the introduction. 79 articles were identified and used to guide the conception, design, and start-up phases of the study.

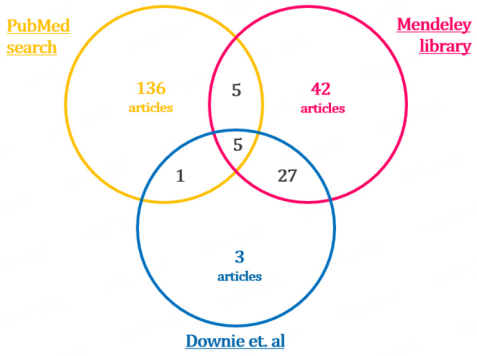

Figure 1. Venn diagram that shows the different sources of the articles.

Figure 2. Grouping of the papers' categories.

Downie *et al.*'s systematic review "Principles of Genomic Newborn Screening Programs: a systematic review" published in 2021 was considered the benchmark and the 36 final articles were included in our initial database[21].

The three sources all together yielded 262 articles, some of which were duplicated in two or all the sources, as shown in Figure 1. The final articles to be screened were 219.

Documents selection

The selection of articles to be included in the review followed two steps, both of which were performed independently by two people:

(1) Titles screening

The first screening was made considering the title of the articles. Articles focusing on one disease only, carrier screening, case reports, protocols only, and infective outbreaks in the Neonatal Intensive Care Unit (NICU) were excluded. After this first step, 151 articles were excluded for relevance reasons.

(2) Abstracts screening

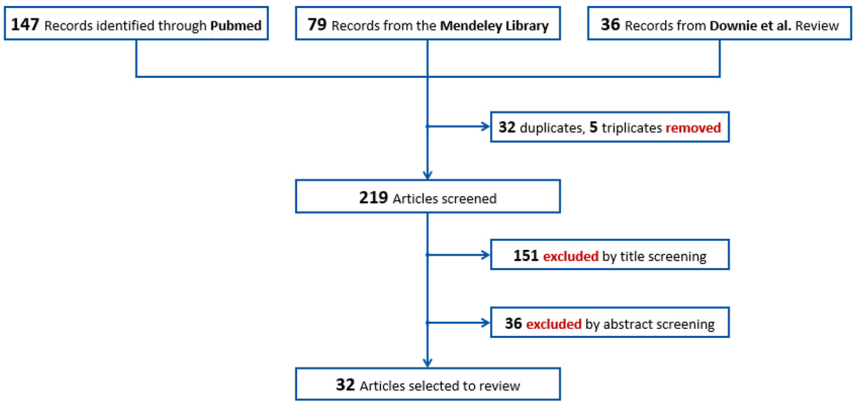

Figure 3. Study flowchart.

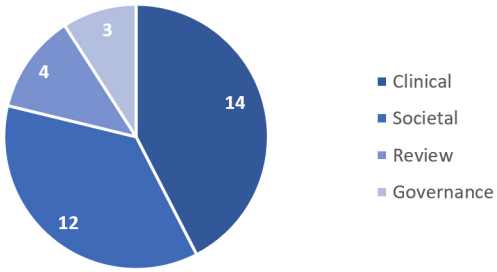

Figure 4. Distribution of the selected articles in the studied areas.

The second step consisted of reading the abstracts and assigning each article to one of 14 pre-identified categories grouped in three areas: clinical, social, and governance [see Figure 2]. Reading the abstracts allowed a stricter selection of articles with a clear focus on the application of Genome Sequencing (GS) to population-wide NBS, while excluding the publications that used GS as a diagnostic tool. Finally, we reduced the redundancy based on the article's topic and publication date (e.g., for articles on the same topic, the most recent was preferred). After this step, 36 articles were excluded.

The final number of articles retained for the review was 33.

A flowchart of the selection of the articles can be found in Figure 3.

RESULTS

The mixed methods search brought to the identification of 33 articles distributed as in Figure 4.

For the Clinical subject, 14 publications were identified. Five papers were focused on wide GS discussion in the last 15 years[22-24,7]. Methods to manage the genomic data produced in the GS analysis and a definition of the clinical actionable conditions have been explored in three publications[2,6,5]. Results and/or discussions about the impact, feasibility, benefits, and costs of the GS in the clinical care of newborns have been reported in five publications[14,26-29].

For the Social subject, 12 publications were identified. Three records dealt with the BabySeq Project, surveying parents and clinicians involved in the trial, parents who denied participation, and a third one analyzing the changed protocol and the concept of family benefit[8,12,30]. The study NC-NEXUS was also taken into consideration, with a publication regarding a Decision Aid tool to support parents in the decision-making process. If GS is to be implemented in NBS, communication and education are key elements that must be considered and promoted[31]. Opinions from genetics professionals were also considered through a paper that presented a survey to the American College of Medical Genetics and Genomics (ACMG) members[32]. Lastly, public views on the incorporation of GS in NBS were also included[33]. Recommendations by the NSIGH Ethics and Policy Advisory Boards were also a result of the search, sharing their opinion regarding the use of GS applied to diagnostic and universal NBS[34]. Finally, two independent opinions and a parents survey were considered[35-37].

For the Governance subject, three publications were identified[9,38,39]. Two[9,38] have a legal focus, analyzing the constitutional framework for the adoption of GS-based NBS programs in the US. The third paper[39] has a policy perspective and lists eight recommendations for the introduction of GS in NBS. These recommendations were elaborated by the Pediatric Task Team of the Global Alliance for Genomics and Health.

Clinical

Wilson and Jungner originally defined the screening criteria to guide the selection of conditions that would have been suitable for screening. Among these criteria, early-stage detectability and treatment availability are still solidly respected. However, the advent of the genomic era with advanced medical technologies and the increased interest in genome screening requested a revision of Wilson and Jungner screening criteria[7]. Certainly, the screening criteria should be further and constantly discussed to reflect people's evolving interests and needs.

The clinical utility of genetic testing and the efforts to guarantee transparency and quality of the results have been widely discussed in Europe and the USA. The Public and Professional Policy Committee (PPPC) and the Quality Committee of the European Society of Human Genetics (ESHG) addressed these challenges in the past years, and the final recommendations were approved and published in December 2012[24]. Whole-genome analysis might be applied in several circumstances, such as diagnosis in symptomatic patients, research, pharmacogenomics, investigation in pre-symptomatic patients, and population screening programs. In order to develop best practices in implementing WGS/WES into health care:

(1) Stakeholders from different fields should participate in the discussions about WGS/WES implementations, sharing their experience and contributing to the development of national and international guidelines;

(2) A targeted approach should be adopted to avoid unsolicited findings, e.g., known genetic variants with limited or no clinical utility;

(3) WGS/WES analysis should be applied when necessary, ensuring the balance of benefits and limitations for the patient. Genetic experts should explain the benefits and limitations of genetic testing for screening, informing prospective parents and raising public awareness;

(4) A protocol is essential to guide the communication of secondary findings and report how the data will be shared and stored;

(5) Guidelines for informed consent on genomic testing, sample uses (e.g., research studies) and storage need to be developed and widely shared within the appropriate workforce;

The European initiative EuroGentest was established by the European Commission to promote accurate and high-quality genetic diagnostics across Europe, and it was integrated as a working group with the European Society of Human Genetics (ESHG), with whom in 2016 they published the guidelines for diagnostic applications of Next Generation Sequencing (NGS) for rare genetic diseases, consisting of 38 statements with a particular focus on WES and sequencing on selected genes identifying small germline variants (Single Nucleotide Variants (SNVs) and insertions/deletions). In 2021, an update of EuroGentest guidelines for NGS has been published, including five additional statements (a total of 44 statements) by the Solve-RD, a Horizon2020-funded project, born with the aim of finding a diagnosis for a large number of rare diseases (www.solve-rd.eu)[22].

GS-based NBS pilot projects

The implementation of GS in newborns triggered great interest in the setting of explorative pilot projects to assess medical, economic, ethical, and social impact in the healthcare system and among the general population.

The BabySeq project (ClinicalTrials.gov Identifier: NCT02422511) is a randomized trial on newborns with the aim to assess the impact of genomic sequencing in the newborn period to screen healthy infants for current and future health risks and provides data about the feasibility, risks, benefits, and costs of the integration of exome sequencing in the clinical care of newborns. The BabySeq2 Project (ClinicalTrials.gov Identifier: NCT05161169) is currently in the recruitment phase and aims to expand and improve the results obtained in the first study. Results reported for the BabySeq project were obtained by the clinical trial on 159 children from the well-baby nursery at Brigham and Women's Hospital (127 healthy newborns) and from the neonatal and pediatric intensive care units at Boston Children's Hospital in Massachusetts General Hospital (32 ill newborns)[28]. 1,514 genes [Supplementary List 1] were curated and classified into three categories (A, B, or C). Category A includes genes with definitive or strong evidence to cause a highly penetrant childhood-onset disorder; Category B includes genes based on actionability during childhood; Category C includes genes that did not meet criteria to be returned in the newborn genome sequencing report[6]. A table including an example of genes from category A from Ceyhan-Birsoy *et al.* (2017) has been appended[28] [Supplementary Table 1].

After testing, a newborn genomic sequencing report is generated, including information on pathogenic and likely pathogenic variants, monogenic disease variants, recessive carrier variants for childhood-onset or actionable conditions, and pharmacogenomic variants. The analysis also contains information on variants of uncertain/unknown significance (VUS) indications. However, only a randomized group of families received newborn GS reports and the results obtained from the study were disclosed to the newborn's parents during an in-person consultation by a genetic counselor and physician. The reports are available in both hospitals and online through a GeneInsight Clinic instance[14].

In the BabySeq project, WES analysis uncovered the risk of childhood-onset diseases in 15/159 (9,4%) of newborns, and none of these was expected based on the clinical histories of babies and their parents. Only parents of 85/159 newborns accepted to receive information on adult-onset actionable conditions, and in 3/85 cases a risk was identified. 88% of newborns were carriers of recessive disease and 5% were carriers of pharmacogenomics variants. Among the newborns with carrier-status variants, 8 of 140 (6%) also had VUS in one of the reported carrier genes. The number of carrier-status variants ranged from one to seven

variants in a single newborn[28].

Regarding the yield of the GS approach compared to standard NBS methods, the BabySeq project's results were discordant compared with conventional NBS and NBS plus WES[29]: 84% of newborns were NBS and WES negative; 1/159 infants were positive for the same disorder by both approaches; 9/159 infants were NBS positive and WES negative. Among the latter, 7 were reported as false positives after subsequent analysis. 15/159 infants were WES positive and NBS negative, indicating the risk of genetic conditions not detectable through the conventional NBS approach[29]. However, the BabySeq project results demonstrated the efficacy of newborn GS in detecting risk and carrier status for a wide range of disorders that cannot be detected by current NBS assays[28].

The North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS) project (ClinicalTrials.gov Identifier: NCT02826694) was concluded in 2020 and examined the use of WES for NBS versus the conventional NBS approach. 106 infants were enrolled, including two cohorts: 61 healthy infants whose parents were approached for participation in the study prenatally and 45 ill infants affected by inborn errors of metabolism (17) and hearing loss (28), already detected by conventional NBS methods. Trio analysis was not performed. However, a follow-up parental sequencing has been performed in cases for which compound heterozygosity was suspected.

In the NC NEXUS project, WES correctly identified 88% of the cases with already diagnosed metabolic disorders and only 18% with already diagnosed hearing loss. Moreover, actionable findings that would not have been revealed by conventional NBS were revealed in four newborns. Some parents were selected to receive additional information about childhood-onset conditions with low or no clinical actionability, clinically actionable adult-onset conditions, and carrier status for autosomal-recessive conditions[27]. Carrier findings in newborns whose parents requested this information were detected with an average of 1.8 per infant (with a maximum of 7 variants).

Clinical actionability was detected using the age-based semiquantitative metric[5].

Conditions were categorized into four categories:

(1) Pediatric conditions with high medical actionability;

(2) pediatric conditions with low or no medical actionability;

(3) adult conditions with high medical actionability;

(4) adult conditions with low or no medical actionability.

According to these criteria, 755 gene-disease pairs were categorized (the list of 755 genes from Milko *et al.* (2019) has been included [Supplementary List 2][5]. An abnormal or positive screen GS-NBS result related to high medical actionability conditions was reported by observing likely pathogenic and/or pathogenic variants in genes associated with pediatric conditions. A normal or negative GS-NBS result was defined by the absence of likely pathogenic or pathogenic variants. Positive results were associated with the presence of likely pathogenic or pathogenic variants found in gene(s) reported in the metabolic or hearing loss diagnostic list. Inconclusive results included, for example, a single heterozygous variant found in a gene associated with an autosomal-recessive condition and/or variants of uncertain significance (VUS) in genes

on the diagnostic list. Negative results indicated no detection of any pathogenic or likely pathogenic variants or any VUS on the diagnostic gene lists. 15/17 (88,2%) of patients affected by metabolic conditions resulted as GS-NBS positive. In the hearing loss cohort, "inconclusive" findings, not providing definitive results, were reported (some participants were heterozygous or homozygous for different VUSs in genes associated with hearing loss). Two false negative results were detected: one patient had a single heterozygous pathogenic variant in a gene associated with maple syrup urine disease and a patient with Malonyl-CoA decarboxylase deficiency had a homozygous missense VUS. However, since the authors did not have sufficient information to better identify the genetic etiology of the patient's disease, both were reported as "inconclusive findings". One patient was a carrier for another condition. 5/28 (17,9%) patients affected by hearing loss tested GS-NBS positive and two of them had positive screen results unrelated to their condition[27].

After the conclusion of the NC NEXUS project, it has been stated that using a GS approach could not widely substitute current screening tests. However, genomic information could be useful to perform a "secondary" or "indication-based" analysis, improving the sensitivity and specificity of NBS for inborn errors of metabolism[27].

In the Netherlands, the NBS (NGSf4NBS) project is a technical feasibility study also aiming at assessing the ethical, legal, social, and financial aspects to explore the adoption of NGS approaches as a first-tier method in NBS[26]. The study will proceed in three steps. In Step 1, inherited metabolic disorders eligible for NGS as a first-tier test will be identified based on treatability. In Step 2, the feasibility, limitations, and comparability of different technical NGS approaches and analysis workflows for NBS will be tested. In Step 3, the results will be incorporated into the current Dutch NBS program, including guidelines for the referral of a child after a positive NGS test result[26].

Methods to evaluate the criteria for inclusion of genes in GS studies

NBS through WGS and WES should be based on a clear path of clinical utility and/or actionability[23]. The magnitude of the genomic information generated, and its management are key challenges of introducing GS in the clinical setting. Other issues that must be taken into account are the definition of a subset of clinically actionable findings, the use of standardized protocols, and the introduction of appropriate and shared informed consensus for the families involved. In 2016, Berg *et al.* defined a semiquantitative metric for evaluating clinical actionability by assessing five criteria: the severity and likelihood of manifesting a particular condition, the efficacy and acceptability of the intervention, and the overall knowledge base of the gene-disease association[2]. The metric did not take into account the individual's age and sex, the timing of the onset of the disease, and the availability and cost of any preventive strategy.

The North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS) project implemented the semiquantitative metric and assessed an age-based framework for evaluating genome-scale sequencing results in NBS. The age-based, semiquantitative metric categorized gene-disease pairs into groups based on age of onset or timing of interventions, improving the past method and facilitating the definition of inclusion criteria in the GS studies[5].

Additionally, a list of genes with putative pediatric relevance based on the framework released by the Clinical Genome Resource (ClinGen) working group has been assessed to manage the return of results in the BabySeq project. The generation of the gene-disease pair association was curated for the following criteria: validity of gene-disease association, age of onset, penetrance, and inheritance pattern. Based on the selected criteria, three categories of classification of gene-disease pairs were defined: category A: genes

included in the newborn genomic sequencing report with definitive or strong evidence to cause a highly penetrant childhood-onset disorder; category B: genes included in the newborn genomic sequencing report based on actionability during childhood; category C: genes that did not meet criteria to be returned in the newborn genomic sequencing report[6].

A comparison between the NC NEXUS age-based framework and the BabySeq categorization approach revealed differences in the methods used to define each category. The NC NEXUS age-based semiquantitative metric includes several components to achieve actionability score criteria, whereas the BabySeq criteria differ between each of the three categories. BabySeq category A is focused on clear evidence of gene-disease relationship without actionability considerations. Category B includes potential actionability. Category C includes low penetrance, insufficient evidence or late-onset conditions, and non-invasive intervention in childhood. A solution proposed was to report actionable genomic information at the corresponding age-appropriate stage (e.g., infancy, childhood, adult) to overcome any potential social, ethical, or psychological issue related to non-actionability conditions[5].

Societal

Incorporating WGS/WES into population-wide NBS programs triggers significant ethical and policy concerns, as it implies the generation of incidental health information of known and unknown clinical significance for millions of infants annually[36]. When implementing a new technology in a state-run program, it is particularly important to reach clarity in the evaluation of benefits and limitations. This is notably valid when the technology is GS, as test results present a heterogeneous, complex, and unsure nature[33].

Conventional (biochemical-based) NBS is considered a standard of care and is often a mandatory, state-supported activity, e.g., in Canada and the US, where parental consent is typically implied[40,41]. Introducing NGS technologies could dramatically change the context, shifting the balance between clinical benefits and risks and raising new questions that could threaten the universality and moral authority of NBS. GS technology has raised fundamental challenges to the traditional ways genomic information is communicated. If GS was to be incorporated into standard NBS practice, clinicians, public health officials, and other stakeholders would need to agree on the type of information that they should seek and communicate to parents[31].

Ulm *et al.* in 2015 surveyed members of the American College of Medical Genetics and Genomics (ACMG) to gather genetic professionals' opinions regarding the use of WGS in NBS[32]. Starting from the premise that 86% of the respondents believe WGS should not be included in NBS yet, many critical challenges were identified, such as the introduction of pre- and post-counseling, the interpretation of results, and follow-up access. Informed consent should be required from parents to enable them to decide which information to receive but with the confidence of knowing that laws and policies are being implemented to protect against discrimination and privacy[32]. It is interesting to notice that at the time the participants filled out the survey (November-December 2012), 28% believed WGS would have been implemented in 5 years (by 2017) and 23% in 6-10 years (by 2018-2022).

Informed consent and return of results

Given the nature of NBS, for which the primary beneficiary is the newborn, parents have a substantial role in the process. Joseph *et al.* conducted four focus groups with socioeconomically and ethnically diverse pregnant women to examine their views and perspectives regarding the potential application of WGS to NBS. For many women, knowledge and information are fundamental tools to have a sense of control over

labor and childbirth - and consultations and education regarding NBS are key topics of conversation that should happen before the test, in order to understand the process and have the opportunity to ask questions[37]. Formal permission or written consent was, however, a secondary priority for parents, while it was felt more urgent in case NBS was performed with WGS, given the increased complexity of genetic information. The need for formal parental permission implies the possibility that parents opt out, thus altering the universality principle that characterizes NBS[34].

Genetti *et al.* in 2018 evaluated parental interest in a randomized trial of GS-NBS, in particular analyzing causes for declining participation, before and after an enrolment meeting with a genetic counselor. Risk communication was found to be a key element during the education process for informed consent, given the sensitivity of genetic information and the apprehension that this information would be recorded in their infants' medical documents[30].

Psychological distress

Families and professionals involved in newborn genetic screening are challenged with complex and onerous questions that can lead to an increased amount of new knowledge which can be difficult to deal with. Parents have the authority, both legal and moral, of making decisions for their newborns, including medical decisions that are, supposedly, in their child's best interest. When using GS, a large number of gene variants are possibly detected, including genes encoding for adult-onset disorders. Such timing of testing, being in the neonatal period, makes it impossible for the primary beneficiaries, i.e., newborns, to make their own decisions depriving them of future adult autonomy and confidentiality[11,12,35,37].

While the use of GS as a diagnostic tool is accepted, the uncertainty and ambiguity of some results of GS as a screening tool could transform healthy newborns into pre-sick or "patient-in-waiting"[42], risking premature medicalization of infants and causing significant distress and worry in parents[34].

Many other potential drawbacks for the screened family are the damage to the child's self-esteem, stigmatization, and the sense of guilt of transmitting a pathogenic variant to your child; this information could also be the cause of discrimination, lack of privacy in different circumstances, with issues accessing medical insurance being the first difficulties on a potentially long list[35].

Genetic professionals and laboratorians are also suffering from potential moral and ethical dilemmas: Ross *et al.* in 2019 reported a case in the BabySeq project where the discovery of an actionable adult-onset disease in a newborn led to a dilemma of the personnel that could not return a result that was widely considered actionable[12]. On the basis of this case, the BabySeq protocol was then modified, invoking the principle of family benefit, for which the best interest of the child includes his parents' well-being. Following these modifications, parents could decide whether they wanted to receive information on adult-onset variants, even though it is still widely accepted[43] that children should not be tested for adult-onset conditions. For Ross & Clayton, one solution could have been to modify the BabySeq analytical process in order not to discover those variants, designing the study to limit the search to relevant genes and reduce the risk of finding stress-inducing information[12].

A survey conducted by Pereira *et al.* published in 2019 demonstrated that parents and clinicians would prefer NBS without GS, even though parents showed more trust than clinicians towards GS. This shows that what is considered a clinical benefit to the clinicians is different from the perception of the parents (i.e., parental/personal utility), which might have a broader range of expectations, showing once again how relevant and crucial the education process is in these circumstances[8].

Considering how fast today's society evolves and how complex and sensitive this field is - more frequent societal consultations are key to understanding whether there is a community consensus.

Governance

The psychological distress and worry around using GS in NBS bring governance and policy consequences that must be taken into consideration. For example, parental worry could cause follow-up visits, tests, and services that may not be medically indicated[36].

Moreover, when clinicians or other healthcare professionals have the role of returning results to patients, time management is a concern, since counseling parents and educating them on procedures and next steps will be time- and energy-consuming and, therefore, costly. It has to be taken into consideration that all positive screen results will need follow-up care, confirmatory testing, and monitoring, ensuing even more time and costs to the healthcare system[34].

Genetics professionals surveyed by Ulm *et al.* think that the complexity implied in the use of GS in NBS should lead to a new counseling paradigm, forcing a non-mandatory program that envisages consent and the option to opt out in a setting where genetic discrimination is prevented[32]. These changes and challenges should thus require a new setting and an infrastructure boosting education and training of the workforce involved[35].

On the same line, two papers[9,38] analyzed the US legal framework with respect to the introduction of GS in NBS programs. Both concluded that the current "constitutional boundaries" do not allow the introduction of mandatory neonatal screening programs using GS. The first argument is that mandatory screening is based on two fundamental legal bases:

(1) *Police power* that allows the state to intervene in order to protect the health and safety of citizens AND

(2) *Parens patrie* that allows public authority to make decisions in the best interest of the children despite the opinion of the parents.

Both principles do not seem to be applicable to genomic screening unless it is limited to a strict number of genes (and variants on those genes) that cause severe but treatable conditions with an almost certain pediatric onset[9,38,39].

From a health policy perspective, there is a consensus regarding the introduction of GS-based NBS programs which should not substitute the current conventional NBS programs, meaning that the costs for implementing the new program are on top of the existing one with limited overlap[39]. Another important aspect considered by all the three papers[9,38,39] is equity: despite being subject to consent from the parents, once introduced, GS-based NBS should be equally accessible to all newborns. An interesting concept linked with equity concerns is the possibility for the families to have raw data from GS analyzed and interpreted independently; if families can get access to raw data, some of them, the wealthier and more educated, could look for deeper analysis and interpretation even for a portion of the genome not included in the NBS program. Is that ethical? Is that fair, considering that other families will not have that possibility?[9]

LIMITATIONS

The rapid evolution of the field and the increasing number of pilot programs using GS for NBS make it difficult to give a snapshot without the risk of missing the most recently published evidence. To make an

example, while preparing this manuscript, a rapid evidence review on the implementation of large-scale genomic screening was published by Alarcón Garavito *et al.*[44].

Moreover, the decision to focus exclusively on NBS programs using WES or WGS forces to neglect some works on disease-specific genetic screening that could provide some additional evidence, especially on topics such as acceptability by the parents and management of incidental findings and VUS.

Finally, for this work, only peer-reviewed articles were taken into consideration. This could have limited the identification of relevant information, especially on governance and legal aspects that could have been included in grey literature, such as project public deliverables, reports, and policy guidelines.

CONCLUSIONS

Although there is a broad and animated debate on the use of GS for NBS, there is still little real-world evidence available from a few pilot projects (namely BabySeq and NC-Nexus, both carried out in the USA). Other pilot projects have been recently launched in Europe and the UK and more evidence will become available in the coming years. Despite a consensus in the literature on the key principles that should guide the use of GS in NBS, many important issues are still to be adequately addressed and solved.

All authors agree that NBS should include only actionable genes, but the definition of actionable is still a matter of debate, as well as the criteria and ideal frequency of updates of the list of genes-diseases to be screened for. Currently, informed consent from the parents seems to be the preferred approach, but there is still an open discussion on how to manage incidental findings or information on the status of the carrier.

Ethical, legal, social, and budgetary issues still constitute great challenges and major barriers to the wide, equitable, and uniform adoption of GS in NBS. When looking at these aspects, it is important to also consider the other side of the coin, i.e., the burden that inherently accompanies a family who did not get the chance of an early diagnosis or the management of critically ill patients in NICUs. Early diagnosis could also generate cost savings for the healthcare systems as it allows them to prevent severe symptoms that may require frequent hospitalizations. These savings could at least partially balance the additional costs generated by GS-NBS, which, according to the majority of authors, should not substitute the current NBS programs but run in parallel as additional screening. Unfortunately, it was not possible to find any published studies with information on cost-effectiveness and the estimation of potential savings of healthcare resources by using GS in NBS.

The management of genomic data of newborns for secondary use (e.g., for research purposes) should be balanced with the right of children to an "open future" and to autonomously make decisions on the use of their own genomic profile. As shown by this literature review, no easy or straightforward solutions have emerged so far. Moreover, a one-size-fits-all approach will probably never work, as GS-based NBS should take into consideration the specific value and ethical frame of the community where it is deployed. Ten years ago, 50% of the surveyed experts of the ACMG expected GS to be implemented in the NBS everyday practice. Evidently, we are not there yet. Further pilots and consultations with the stakeholders will be necessary before GS-based NBS programs can be widely implemented.

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Authors' contributions

Design of the methodology and the search algorithm: Magnifico G, Benvenuti S
Blind selection of the articles: Magnifico G, Artuso I
Writing of the manuscript: Magnifico G, Artuso I, Benvenuti S

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Opinion

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Could federated data analysis be the catalyst accelerating the introduction of newborn genome screening for the detection of genetic disease?

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Abstract

Data federation intermediated through trusted research environments can help accelerate the adoption and utilization of newborn genome screening worldwide. Data federation will protect individual datasets from unauthorized security breaches, allow analysis *in situ*, and bypass the need for cumbersome data sharing agreements between parties. Finally, data federation could accelerate the adoption of new therapies for rare genetic diseases with the use of synthetic clinical trials.

Keywords: Newborn genome screening, data federation, trusted research environment

INTRODUCTION

Worldwide, millions of children are born with a rare genetic disease[1,2]. Newborn screening (NBS) has been effective in identifying babies who are at risk of developing a genetic disease and initiating a therapeutic intervention. The first genetic disease for which NBS was introduced is phenylketonuria (PKU), where early

dietary intervention prevents serious mental deficiency[3]. In the past fifty years, mandated NBS has expanded to include other, mostly Mendelian, diseases where early therapeutic intervention has been effective in preventing and/or ameliorating irreversible tissue damage. In many countries, states, and regions of the world, public health programs are in place to collect blood specimens from babies soon after birth[4]. Analytes extracted from dried blood spots collected on filter paper are assayed using gas chromatography/mass spectrometry (GC/MS) or tandem MS.

A second layer of screening based on Next-Generation Sequencing (NGS) technology could expand the scope of the existing NBS programs[5,6]. This additional layer of screening will not replace what is currently used, but it will increase the current offering substantially to include a broader spectrum of disorders not detectable by tandem MS.

Newborn genome sequencing could evolve to become the new paradigm for healthcare delivery, where early detection could result in better clinical outcomes. Rapid Whole Genome Sequencing (rWGS) has been shown to be an effective diagnostic test linked to decreased infant mortality and improved outcomes in babies admitted to Neonatal Intensive Care Units (NICU)[7,8].

Extending the use of genome sequencing as a screening test to all newborns is only a matter of time. However, before newborn genome screening is widely adopted, several factors will need to be carefully considered, including:

1. Accurate definition of pathogenic genomic variants in diverse populations.

2. Defined care paths for the follow-up of a screen-positive finding.

3. Evidence that early intervention leads to improved clinical outcomes.

4. Detailed cost analysis.

Persuasive answers to the above will be required by the key stakeholders whose support is essential, i.e., parents, health care providers, public health policymakers, and the pharmaceutical industry.

Several newborn genome screening (including whole genome sequencing and whole exome sequencing) initiatives have been launched, or they will be launched soon[9-11].

We anticipate that no one project will have the necessary solutions to satisfactorily address all or some of the above-mentioned problems. Thus, aggregation of information collected from different sources could provide part of the solution for critical mass and momentum.

Data aggregation of such magnitude presents significant legal, ethical, and technical challenges related to (i) the security and privacy of sensitive information; (ii) the size and varied nature of stored genomic data; and (iii) legal requirements for data sharing. A viable near- and mid-term solution that can help address these issues will be using trusted research environments (TREs) and data federation for secure storage, access, and analysis of genomic data[12]. A comparison of risks and benefits between existing and federated databases for genomic data is shown in Table 1.

Table 1. The data aggregation challenge. Comparison of risks and benefits between existing and federated databases

	Databases	Federated databases
Security and compliance	Movement and copying of sensitive information increases the risk of data breach	In a TRE and federation environment, data are not moved or copied, reducing security risk
Data size and interoperability	Lack of standardized formats and pipelines limits interoperability, and negatively impacts scalability, cost, and efficiency	Fully standardized data, securely accessible by cloud-based platforms through federation, can be combined with global cohorts and disparate datasets
Collaboration	Data cannot leave jurisdictional borders. Data sharing agreements are frequently difficult to negotiate and implement, hindering collaboration	Federated approaches will eliminate a major barrier across individual datasets, vastly improving the statistical power of research

TRE: trusted research environment.

Federated data analysis platforms, which facilitate secure data access from multiple sources without the need for data movement- where data could be vulnerable to interception, have emerged as a promising part of a solution for safely sharing anonymized genomic data. Here, genomic data remains secure in the TRE, which can then be linked virtually using a set of Application Programming Interfaces (APIs).

Traditional data access methods involve researchers downloading data to an institutional computing cluster. With federated analysis, the analysis is brought to where the distributed data lies, thereby eliminating the risky movement of data and removing many existing barriers to accessibility[13]. Such technology means that data can be made securely accessible but that data controllers (e.g., biobanks and healthcare providers) retain jurisdictional autonomy over data, a key concern in international data sharing.

International initiatives such as the Global Alliance for Genomics and Health (GA4GH)[14] set standards to promote the international sharing of genomic and health-related data, in part by setting interoperability standards and providing open-source APIs.

Common Data Models (CDMs) are crucial to ensuring data is interoperable, with several growing in popularity in the life sciences sector recently, including OMOP (Observational Medical Outcomes Partnership) CDM from the OHDSI (Observational Health Data Sciences and Informatics)-specifically for clinical-genomic data. Examples of health organizations utilizing OMOP as their CDM include the UK Biobank and All of Us from the US National Institutes for Health (NIH)[15,16].

Additionally, extraction, transformation, and loading (ETL) pipelines that can automate this work to process and convert raw data to analysis-ready data help further simplify this process for researchers. Normalizing all data to internationally recognized standards allows researchers to perform joint analyses across distributed datasets, which is key to ensuring diversity and representation of as many populations as possible in studies.

These standardized and interoperable datasets could be combined seamlessly for analysis via federation, enabling researchers to analyze this data collaboratively in conjunction with other complementary datasets. Standardization of data formats and analytical approaches within and even between health systems can bring substantial benefits in terms of comparability of data and contribute to continually improving processes.

Illustrative examples with potential multiplier effects could include:

Sharing pathogenic variants: Defining the frequency and prevalence of a pathogenic variant in diverse populations is essential. Access to the pathogenic variant libraries of the various initiatives will impact the predictive value of a screen positive, and it might help in the reclassification of Variants of Uncertain Significance (VUS).

Sharing care paths: Newborn genome screening is a risk stratification test that places a person in a high- or low-risk group for a particular genetic disease. The accuracy and validity of establishing the presence of disease have an enormous impact on the well-being of the person and the family, the timing of therapeutic intervention, possibly the modality of intervention, and ultimately healthcare cost.

Sharing clinical outcomes: Managing individuals with latent or early-stage disease can potentially increase the burden on health care providers and the health care system. Therapeutic interventions, to the extent possible, will need to be evidence-based. Individual genetic illnesses are often uncommon, and randomized clinical studies are difficult to conduct. Sharing clinical outcomes, on the other hand, may provide an incentive for synthetic clinical studies.

Sharing the analytic modality used to generate a variant: The validity of a variant is frequently linked to the analytical platform used to generate the information, i.e., panel, short-read NGS, and long-read NGS. Providing a barcode record could be helpful in assessing a variant and its possible value as a biomarker.

Recently, a pioneering example of a multi-party federation between Genomics England and Cambridge Biomedical Research Centre (BRC) was demonstrated. This allowed secure data analysis across TREs in the UK's first known demonstration of genomic data federation. This highlights that the technology now facilitates secure data access via federation for authorized researchers to perform joint secure data analysis on global cohorts. Data sharing via federated databases also decreases the danger of unauthorized access and encourages the adoption of advanced privacy-preserving encryption methods when analyzing data[17]. It is conceivable to imagine that this technology could be used in an undiagnosed disease program targeting newborns to help the integration of clinical, genomic, therapeutic, and outcome inputs residing in different datasets. A summary of how this could work is provided in Figure 1.

The Federated European Genome-Phenome Archive (EGA) is another program that uses federation to provide global discovery and access to human data for research while still adhering to jurisdictional data protection rules. The Federated EGA promotes data reuse, facilitates reproducibility, and accelerates biomedical research by providing a solution to increasing issues in the safe and efficient handling of human omics and related data[18].

While there are significant advantages to moving towards a genomic approach to newborn screening, these programs also have challenges. These include considerations of the ethical, legal, and social implications (ELSI) of newborn genomic screening - these can include concerns surrounding sensitive data sharing, patient autonomy, and consent. A detailed discussion of these issues is out of the scope of this piece, but we refer the reader to other references that have discussed these issues more completely[19,20].

As federation is an emerging technology, careful consideration must be given to scaling up federation across different TREs, particularly surrounding governance and assurances in particular across different jurisdictions. Additionally, genomic data federation could potentially have risks, which may include improper use of data, hacking, and identification of incidental findings such as detection of variants associated with pathologies not immediately treatable or relevant to the newborn[21]. It is important that

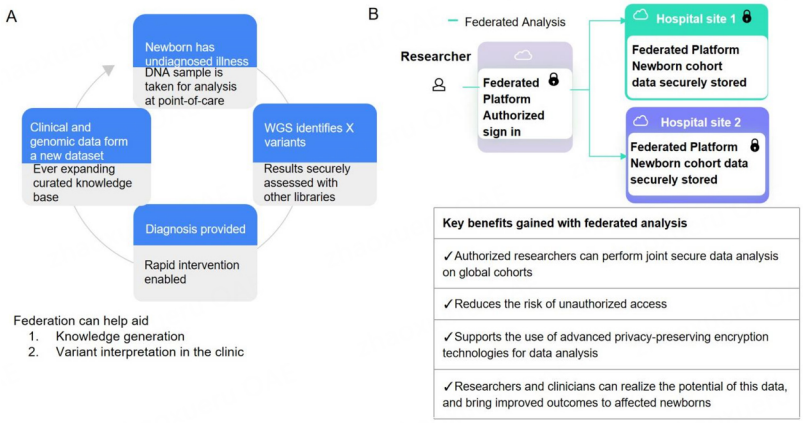

Figure 1. An overview of how federated data analysis can be incorporated into an undiagnosed disease program targeting newborns to help enable secure data access across research laboratories and clinics worldwide. (A) The steps involved in diagnosing a rare disease in an affected newborn; (B) A summary of how federated data analysis is performed and the benefits that can be gained.

these all be considered and addressed as federated approaches continue to be developed.

CONCLUSION

Newborn genome screening is a promising approach to early disease detection with considerable advantages compared to traditional approaches, but the integration into clinical care comes with complex technical challenges, which must be meaningfully explored to ensure effective and equitable impact. Standardized data federation could provide part of a crucial solution as a collaboration framework for the various newborn genome screening initiatives underway worldwide. Such efforts to facilitate secure joint data access and analysis to information among relevant stakeholders will accelerate the existing momentum of collaboration between global newborn sequencing initiatives, ultimately improving outcomes for patients.

DECLARATIONS

Authors' contributions

Wrote the paper: Tsipouras P, Sheppard H, Gaimster H
Reviewed the paper: Chatzou Dunford M, Zaoutis T

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Next-generation sequencing-based newborn screening initiatives in Europe: an overview

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Abstract

Aim: This article describes results from a survey targeting healthcare professionals (HCPs) leading newborn screening (NBS) initiatives in Europe. The survey was developed within the framework of a dedicated working group set up by the International Rare Diseases Research Consortium (IRDiRC) to gather collective efforts relating to NBS. The objectives of the survey were to gain a better understanding of approaches being tested for the expansion of NBS and to raise awareness of the significant momentum across Europe to evaluate novel technologies for use in future NBS programs.

Methods: A web-based survey including 57 questions was developed to gather information about genomic newborn screening initiatives in Europe that are using next-generation sequencing (NGS) as a first-tier test. Responses were analyzed qualitatively, and aggregated results are presented herein. The identity of some initiatives is not presented to preserve confidentiality.

Results: The findings of the survey indicated that most initiatives are in the planning stage and have not yet started. Although all 14 studies are heterogeneous in design, there is broad consensus that NGS approaches to NBS will, in the short term, be implemented in parallel with current screening programs. The results of this survey can be used to inform the design of studies still in the early planning stages.

Conclusion: Here, we provide an overview of NGS-based initiatives in Europe. Importantly, the initiatives described herein will generate evidence to evaluate the utility and feasibility of NGS approaches to NBS, thereby shortening the pathway to responsible implementation of NGS in NBS and informing future research efforts.

Keywords: Newborn screening, rare disease, genetic disease, genomic sequencing, genomic screening

INTRODUCTION

NBS is one of modern medicine's most successful public health initiatives. The identification of life-threatening or severely debilitating conditions in the newborn period can enable early treatment and intervention plans.

Traditional NBS with tandem mass spectrometry (MS/MS) has enabled screening programs to effectively test for dozens of conditions at low cost[1-3]. However, current NBS with MS/MS is limited to blood- or urine-based metabolic biomarkers. There are hundreds of early-onset genetic conditions that do not have discriminating metabolic biomarkers with disease-specific interventions and, as a result, are not yet systematically screened. Early treatments are available for many conditions (e.g., pyroxidine-dependent epilepsy[4]), but efficacy is limited if initiation of treatment is delayed beyond the first few months of life, creating a critical need to consider additional NBS approaches.

Technological advancements in high-throughput NGS[5] have allowed NBS programs to consider expanding screening to include disorders without readily accessible biochemical biomarkers. In a diagnostic setting, strong evidence from studies of critically ill infants with signs and symptoms of a possible genetic disorder has already demonstrated the post-natal utility of genomic sequencing (i.e., whole-genome sequencing)[6-12].

Further, there are several studies underway that directly investigate the impact of agnostic genetic testing on newborns. For example, BabySeq is a randomized controlled trial focused on determining the benefits and risks of newborn genome sequencing. In the BabySeq study, newborn genomic sequencing revealed a risk of childhood-onset disease in 9.4% of newborns and reported carrier status for recessive diseases in 88%, noting that none of the disease risks were expected based on the infants' or family histories nor were they detectable by traditional NBS assays[8,13,14]. There is an increasing number of resources and databases with well-curated genes-disease associations and relevant treatment strategies. For instance, in 2021, the Rx-Genes database became publicly available, including 633 conditions for which treatment is now available[15]. A year later, the resource Genome-to-treatment (GTRx) was also made available after a list of 8,889 interventions and over 5,000 publications were reviewed, leading to the retention of 421 disorders for which effective treatments are available[16].

Given the potential of incorporating NGS assays into current NBS programs, numerous large-scale initiatives have been announced across the globe, including the Genomic Uniform-screening Against Rare Diseases in All Newborns (GUARDIAN study[17]), BeginNGS[18] and Early Check[19] in the USA, BabyScreen + in Australia, and Screen4Care[20], Generation Study[21,22], Baby Detect[23] and PERIGENOMED in Europe[24]. To develop the safest and most efficacious NGS-based NBS, it is important to have knowledge of each program's goals, study design, deliverables, and expected impact on current NBS. Thus, the IRDiRC sought to gain an understanding of current and planned NBS initiatives including large-scale and pilot studies by conducting a survey. The specific objectives of this exercise were to gain a better understanding of the variety of approaches being tested for the expansion of NBS and to raise awareness of the significant momentum across Europe to evaluate novel technologies for the future benefit of public health programs such as NBS.

METHODS

A web-based survey, using the free online Survey Monkey platform, was developed by several members of a dedicated working group on NBS set up by IRDiRC to gather information about newborn sequencing initiatives in Europe that are using NGS as a first-tier test. NGS approaches include whole-exome sequencing (WES), whole-genome sequencing (WGS), and/or classic NGS gene panels. First-tier NGS test was defined as the first test to be used for screening newborns for a list of early-onset, severe, and treatable genetic conditions. The survey contained 57 questions inquiring about different aspects of each initiative, including study design and methodology, testing technology, confirmatory testing, test validation, data analysis and follow-up, cost-effectiveness, and vision for the future. The full questionnaire can be found in supplementary materials. A link to the online survey was disseminated via email and responses were analyzed qualitatively. Several of the initiatives requested that their data remain anonymous as they are still in the planning phase and have yet to secure funding for their studies. Therefore, for the purposes of this article, the identity of some of the initiatives is not presented and only aggregated results are presented to preserve confidentiality.

Initially, we planned to distribute the survey to the lead and co-lead investigators of 17 NBS initiatives in Europe in April and May 2023. However, prior to survey distribution, we learned that three of the selected initiatives did not use (or plan to use) NGS for NBS as a first-tier test. Thus, the total number of surveys distributed by email was 14. The IRDiRC NBS working group was asked to compile a list of European NGS-based NBS initiatives and surveys were distributed accordingly via email. It is important to note that our survey pool does not represent a comprehensive landscape review, and that caution should be exercised with regard to the interpretation of survey results.

RESULTS

General information

Respondents

All surveys were completed and returned with one respondent per initiative. Twelve respondents provided the name of their initiatives: Baby Detect, FirstSteps, Genome-wide Screening Pilot Study (GSP Study), Generation Study, GenNatal, NGSf4NBS, Neonatal genomic screening: feasibility, expectations, definition of the diagnostic pathway, and public health implications, PeriGenoMed, PROGETTO GENOMA PUGLIA, Responsible Implementation of Newborn Genome Screening (RINGS), Screen4Care and Shifting Perspective on scReening for Inborn errors of immunity with Neonatal Genetics (SPRING). All initiatives were considered research pilot projects focused on the technical feasibility of selected NGS approaches (i.e., WES, WGS, and/or classic gene panels) in NBS as a first-tier test and are or will be carried out in parallel to the existing NBS programs.

Most laboratories participating in this survey were genetic (6), followed by NBS (3), clinical (2), and immunological (1). Two initiatives were part of a government organization. Six initiatives are ongoing or about to start enrollment, while the remaining eight are still in a preparatory phase. One initiative had concluded the first part of a two-stage study at the time of writing (manuscript in preparation). Several aspects of the research pilots are either yet to be fully defined or subject to change with study progression.

Regional breakdown and catchment/scope

Apart from one pan-European research study with two pilot trials planned in Germany and Italy (i.e., multi-national), all other initiatives are focused within one European country and include three initiatives in

Italy, three in the Netherlands, two in Spain, one in Belgium, one in England, one in Germany, one in Greece and one in France [Figure 1A]. As illustrated in Figure 1B, four of these initiatives are enrolling patients within a single clinical site or maternity ward (i.e., local). Three will be focusing on sites within one region (i.e., regional), while four others will be recruiting from several sites across different regions within their countries (i.e., multi-sites). Two other initiatives will be recruiting from sites within all regions within their countries (i.e., national).

Funding

Only one initiative is supported solely through private funds (i.e., companies or for-profit organizations). Six have secured (or are hoping to secure) public funding (i.e., governmental funding/not-for-profit organizations), and the remaining seven are or will be using a combination of private and public funds.

Engagement with stakeholders

When asked about engagement with stakeholders such as patient advocacy groups and/or members of the public, nine initiatives indicated plans for engagement. For one initiative, patients were consulted prior to the project start to participate in discussions on the definitions of treatability and actionability for disease conditions.

For the nine respondents who confirmed engagement with representatives of patient groups or the public, the level and type of engagement varied. For example, public input was sometimes limited to discussions around ethical, legal, and societal concerns, while others reported aspirations to engage with these groups more broadly. Examples of broader engagement included involving representatives of patient groups either within the steering committee or across all project activities. Another example of broad engagement is illustrated by the organization of a national public dialogue, through representation on the steering group and working groups, and via user research to support program design. In this instance, feedback from the engagement carried out with different stakeholders involved will help inform the design of the NGS-based NBS initiative.

Half of the initiatives have no plans to engage with the national NBS committee (or equivalent authority) of their countries. Seven initiatives have plans to engage including six initiatives that have included a representative of the national NBS committee within the steering or program committee to either (i) oversee the impact of implementation on the current and future NBS program and ensure the quality, accessibility, and affordability of using NGS for NBS; (ii) discuss what evidence would be required to evaluate the program or (iii) simply be informed of the project's progress.

Desired impact on stakeholders

Survey participants hoped to attract the interest of a variety of stakeholders by demonstrating the technical feasibility of using NGS for NBS. Healthcare professionals (HCPs) and policy makers were the two most cited stakeholder groups (cited by 12 and 11 initiatives, respectively), while NBS and other professional societies, ministries of health, and patient advocacy groups were second (each cited by eight initiatives), and finally, the public, cited by three initiatives [Figure 2].

Study design & methodology

Study type

Most initiatives will be exclusively using a prospective study design for patient recruitment (n = 9). Four have opted for a pilot with two arms, including a prospective and a retrospective arm. One initiative will only test a small cohort of patients retrospectively. Overall, retrospective studies planned to recruit fewer

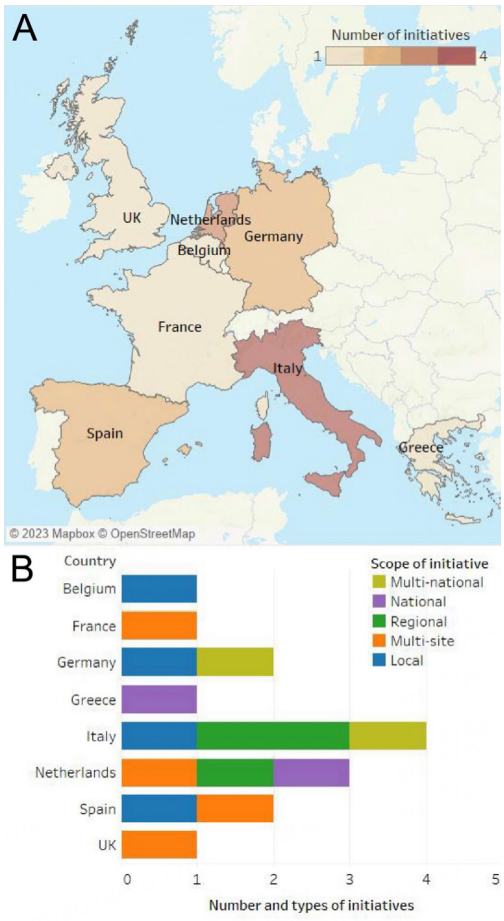

Figure 1. (A) shows a map of surveyed initiatives in Europe. The pan-European study is indicated twice as piloted in both Germany and Italy; (B) shows the scope of initiatives per country.

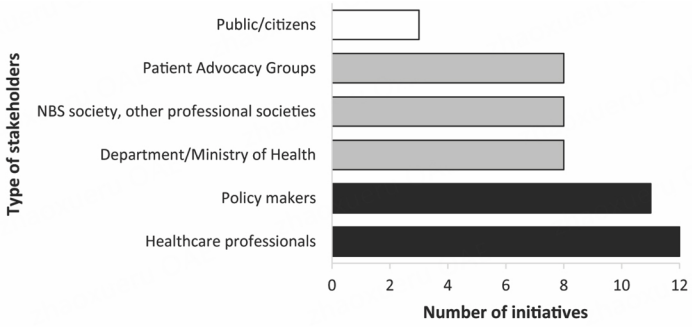

Figure 2. Desired impact on stakeholders.

participants (ranging from 10-100 to 101-1,000) than prospective studies (ranging from 10-100 to 100,000 for the largest initiative).

Parent information, enrollment, and consent

Midwives are expected to be the main recruiting HCPs, followed by nurses and other specialized practitioners including obstetricians, neonatologists, psychologists, genetic counselors, and clinical

geneticists.

Although not all initiatives have confirmed their plans, eight are presently intending to start providing information about genetic testing to expectant parents during the third trimester of pregnancy; five initiatives plan to start providing information earlier in the first or second trimester. Enrollment will start during the second trimester of pregnancy for one initiative and during the third trimester to after birth for the others, with the acquisition of informed consent from parents following a similar timeline. For one initiative involving several sites, the timing of informed consent will vary, offering participating centers the flexibility to adapt their timing [Figure 3].

Sample type

All initiatives will extract genomic DNA from dried blood spots. Two initiatives will test cord blood for the NGS analysis, including one that will add a saliva swab to the sample types to be tested. Thirteen initiatives plan to collect samples upon birth or within 3 days after birth. One initiative focusing solely on the technical feasibility of using WGS for screening will be collecting samples from children of all ages from a disease-affected cohort of patients with an already confirmed molecular diagnosis. These patients will be recruited from the outpatient clinics of the participating University following diagnosis. Another initiative will make efforts to collect samples in parallel with its national NBS program. For all the others, DBS samples will be collected independently of the national NBS programs.

Study duration

Five initiatives have a study duration of up to 12 months and five will be carried out over 18 to 24 months. Two initiatives will last for three years, and one will last for four. For several initiatives, study duration includes preparation of the sequencing workflow and analytical pipeline as well as recruitment, sequencing, and analysis. For other initiatives, the project is broken down into phases, with cohorts increasing in size. One initiative did not provide information related to study duration.

Testing approaches, confirmatory testing, and test validation

The selected NGS approaches vary among the surveyed initiatives [Figure 4].

• Eleven initiatives have selected a single NGS approach for their studies:

1. Six initiatives are using or planning to use only WGS as a first-tier test for NBS, including one that will also be testing parents using WES to facilitate filtering of variants in selected genes.

2. Three initiatives will be using classical NGS gene panels.

3. Two initiatives will be using WES.

• Two initiatives will use a mix of NGS approaches:

1. One initiative is planning to test and compare WES and WGS.

2. One initiative is comparing WES, WGS, and classical NGS gene panels.

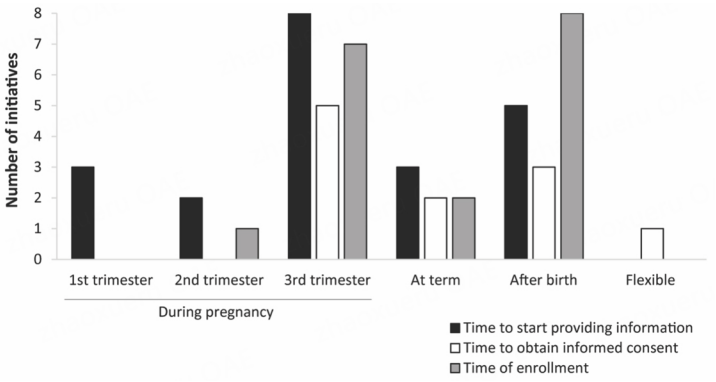

Figure 3. Timing for providing information, securing informed consent and enrollment.

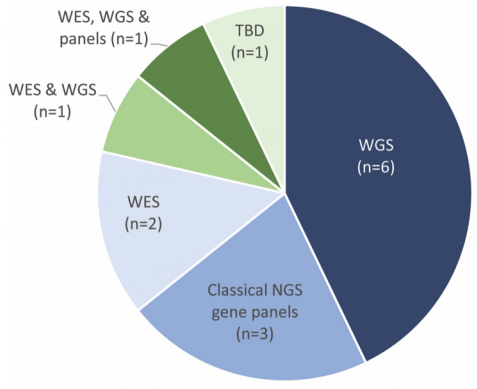

Figure 4. NGS approaches tested in the initiatives as a first-tier test for NBS.

• One initiative has not selected a preferred approach as it is deciding between virtual gene panels through WES or classical NGS gene panels (TBD in Figure 4).

Ten initiatives plan to do confirmatory testing of the NGS test results, although the type of confirmatory tests to be used varies by disease and the strategy employed is dependent on specific genes and variants. For example, some respondents mentioned using Sanger sequencing to confirm the presence of a specific variant identified on NGS or biochemical testing to reveal abnormal enzyme function that could be consistent/inconsistent with the presence of any functionally significant variant in the encoding gene.

Six initiatives are linked to the existing national NBS programs in their respective countries and some of these will use the results from the national NBS program as confirmatory testing for the NGS test for conditions that are currently included in the national program. For other studies, the national NBS programs and NGS initiatives are more loosely connected, with no firm agreement at present on the selected method for confirmatory testing, but with the intent to explore how to monitor false positives and false negatives resulting from NGS tests based on current NBS program results. Eight initiatives are planning to validate their NGS test for its ability to detect a pathogenic variant using one or more of the following: validation through known samples (n = 6), cell lines (n = 2), and in-silico samples/mutations (n = 1).

Disease inclusion, gene lists, and variant types

Two initiatives focus on specific types of conditions: one on metabolic disorders and one on inborn errors of immunity. The remaining twelve initiatives have developed inclusion criteria for disease selection. Ten initiatives have ensured that clinical care pathways are available in their country for all diseases on the screening list, while for the remaining four, care pathways are in place for some, but not for all, diseases to be screened.

Inclusion criteria applied for disease selection

Three initiatives will apply the Wilson & Jungner inclusion criteria for NBS[25], including treatability, disease onset, disease severity, penetrance, and clinical validity [Supplementary Table 1].

Most initiatives, however, will use a modified version of the criteria to enable a larger number of conditions to be screened with NGS, hence the need to add "genetic feasibility" (i.e., conditions with a known genetic biomarker that can be identified by NGS technologies) to the criteria for inclusion. Although all initiatives will screen for conditions that manifest in early childhood, the specific age of onset might vary. One initiative has yet to decide the inclusion criteria for their initiative. In addition, three initiatives have chosen to use two distinct lists for disease inclusion, one for treatable diseases and one for actionable diseases. Although there is not a universally accepted definition for each of these terms, according to the key principles on NBS developed by EURORDIS[26], treatable conditions refer to conditions where early identification helps to avoid irreversible health damage. Actionable conditions, which includes treatable conditions, is a broader term encompassing (1) conditions where early interventions lead to health gain for the newborn; (2) conditions where early diagnosis prevents the lengthy diagnostic odyssey, and (3) conditions where parents will have reproductive options during subsequent pregnancies. Several investigators support the concept of expanding inclusion to diseases affecting young children, but without an agreed and common definition, the variability in disease selection is likely to be linked to the differences in the interpretation of treatability and actionability. Furthermore, there are inherent difficulties in clearly defining what would constitute proof that early intervention leads to improved outcomes.

Based on the agreed selection criteria, the numbers of diseases and genes to be screened vary widely among the initiatives, ranging from 100 different diseases and genes for one initiative to 300-450 different diseases and genes for others. One initiative is planning to screen for over 500 genes [Supplementary Figure 1]. There does not seem to be a relationship between the NGS approach and the number of genes to be included in the screening, although certain NGS tests like WES and WGS will allow easier inclusion of additional conditions and genes as it is possible to filter post-sequencing for conditions and genes of interest[27] [Supplementary Figure 1].

Ten of 14 respondents who have selected WGS and/or WES as the NGS approach(es) have indicated that it will be possible to add or subtract conditions on the disease list during the duration of their initiatives. All agree that disease selection should remain flexible in the future.

According to the classification and guidelines from the American College of Medical Genetics and Genomics (ACMG)[28], all initiatives plan to screen selectively for pathogenic variants and, to a lesser extent, likely pathogenic variants (12 respondents). Regarding the types of variants to be screened for, small insertions and deletions (indels), single-nucleotide variants (SNVs), and copy-number variants (CNVs) are at the top of the list, with structural variants (SVs) and short tandem repeats (STRs) included for some [Supplementary Figure 2].

Data analysis and follow-up

Data analysis and storage

The analytical phase of NGS testing occurs in two distinct stages, referred to as primary analysis and secondary analysis. During primary analysis, raw data is generated by a sequencing instrument. Secondary analysis takes this raw data as input and, through comparison with a reference genome, identifies genetic variants present in the specimen. Following quality control assessment of the results of primary and secondary analysis, the post-analytical phase, referred to as tertiary analysis, begins. Tertiary analysis includes annotation, interpretation, and reporting[29]. For secondary and tertiary analysis of NGS-based NBS pilot data, more than half of the respondents (n = 8) will be using a hybrid solution including a mix of in-house and commercially available analytical tools. Four initiatives have selected commercial software, while one will be using in-house developed bioinformatic tools. Another initiative has yet to be decided regarding this part of the project. Although three are undecided and others may change strategy during the course of their studies, six initiatives have chosen to store data on premises, three will be using cloud-based solutions, while five others will be using both on-premise storage and cloud-based solutions. The type of files to be stored includes, for most, variant call format (VCF) and FASTQ, with a minority also looking at keeping compressed reference-oriented alignment map (cram), special callers, annotated/prioritized variant outputs, and files on quality control. The duration of data storage is not standardized across the initiatives; four initiatives will keep these files for three to four years, while the others intend to store them for longer, with two respondents specifying that they will store data for 10 years to support long-term clinical follow-up.

Return of results

The desired or estimated time from sample collection to results varies widely among respondents, from four days to four months, although a third of initiatives have yet to define this aspect. Apart from three initiatives not seeking to return any results to study participants, seven initiatives aim to return results to families with positive and negative genetic screening results while four will only inform parents of babies with a positive screening result.

Post-service evaluation, data linkage and clinical follow-up

Ten of 14 initiatives plan to recontact the parents of the newborns for post-service evaluation of their participation in the pilot studies at one or more of the following time points: at the end of the study, 3 or 12 months after the end of the initiative, or even within three years after study conclusion.

When asked whether genetic screening results will be linked to clinical datasets in the long term, five respondents who answered positively were largely undecided as to how this linkage will or should happen. Only one initiative has a specific plan to store de-identified genomic sequence data together with ongoing health data in a national repository. This practice will continue until the participant withdraws, i.e., parents withdraw on behalf of the newborn, or at age 16 when the young person will be asked to consent for their data to remain as part of the study.

Half of the initiatives will follow up on clinical outcomes, although how this will be done is not yet fully defined. For one pilot specifically, follow-up will be done with clinicians and families of babies who screened positive to assess clinical outcomes.

Of the seven respondents who answered positively to follow-up on clinical outcomes, four indicated that these clinical outcomes will not be linked to electronic health records (EHRs) or other data sources. However, one initiative has indicated that some outcome data will be ascertained through de-identified health records and included in a national repository. Two initiatives would like to link clinical outcomes to

other data sources either by reviewing records locally or to qualitative and quantitative research with clinical teams and families.

Data federation implies the possibility to combine data from multiple sources to facilitate sharing and pooling of data for analysis. Respondents were asked whether they had considered federating any data from their initiatives. Eight of 14 answered positively, with some arguing that sharing knowledge through a database would help with the rapid interpretation of variants. In addition, data federation would help assess the sensitivity and specificity of the NGS tests for NBS.

Cost-effectiveness and health economics

Twelve respondents will perform a micro-costing analysis of their NGS-based NBS test to understand the operational cost of the workflow. For one initiative in particular, the intent is to compare the operational costs of several NGS approaches, although the investigators have yet to secure funding for this part of the study. When asked whether they would be collecting data on economic utility and if they were planning long-term follow-up of individuals with identified etiological variants, only six respondents answered positively, indicating that they would be using the criteria described in Figure 5 to demonstrate the potential economic value of screening using NGS.

The proposal assessing long-term economic impact is not one that appears to be fully mature for most respondents, with three having yet to define what type of data they will collect for that purpose. Five initiatives are planning to evaluate medical resource utilization through EHRs and one will also try to use health insurance claims to assess the long-term economic impact of the NGS-based NBS. Furthermore, seven initiatives will also attempt to capture cost data in conjunction with healthcare resource utilization data.

For health economic analysis, it is important to describe a comparator group that will act as a control (e.g., a group of individuals that did not receive an early diagnosis through NBS). More than half of the initiatives have not included a comparator group within their initiatives. Among those who have, one initiative is comparing non-participating hospitals with participating hospitals to obtain matched controls by interrogating laboratory and clinical records. Others mentioned that historical cohorts will be used as controls for conditions with a well-known natural history.

Vision for the future

Apart from one respondent who sees NGS-based screening replacing biochemical screening in future national NBS programs, all others believe that genomic screening will be used and implemented in parallel to traditional NBS programs, at least until the sensitivity and specificity of NGS-based screening are comparable to those of biochemical screening for all conditions currently included in national NBS programs.

All the initiatives included in this report are research-driven. Therefore, the impact within healthcare systems will only be tangible once adopted by decision makers and regulatory bodies. Most of the respondents believe that NGS-based screening will be adopted as a first-tier NBS test within the next 10 to 15 years.

DISCUSSION

Increasing numbers of targeted therapies that drive precision medicine coupled with recent advances in genome sequencing technology, particularly reductions in turnaround time[16,30], computational advances for

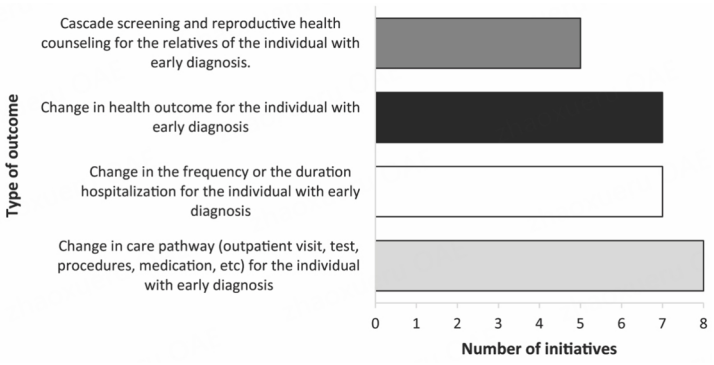

Figure 5. Outcome metrics that will be assessed during the follow-up.

identifying and interpreting pathogenic variants[27,31,32], and reduced sequencing costs[33,34], mean that the next few years will be pivotal in the transformation of NBS as we know it today.

The survey results indicate that most studies are in the planning stage. Although there is heterogeneity in study design across the initiatives surveyed, there is broad agreement that in the upcoming years, NGS-based approaches to NBS will be implemented in parallel with current screening programs. Most envision that NGS will supplement rather than replace current NBS. These initiatives are not only essential to evaluate the utility, feasibility, and acceptability of NGS-based screening in countries with different healthcare systems, processes, and cultures, but they also help to improve our collective understanding of rare diseases by enabling future research and drug development.

Diversity in the choice of and access to secondary and tertiary analytical software is represented within the surveyed initiatives. Not all secondary and tertiary pipelines are able to identify all types of variants. Consequently, there might be limitations in the detection of specific variants depending on the capabilities of the selected analytical software.

The heterogeneity in the design of initiatives extends to decisions about how many and what conditions might be included in an expanded NBS program. In general, there was consensus in using a modified Wilson and Jungner framework, which included concepts such as treatability and actionability. As would be expected, how these concepts were operationalized was dependent on the context of the different national policies and healthcare systems. There are also inherent difficulties in defining what would constitute proof that early intervention leads to improved outcomes. The reality of implementation in a real-world setting is complex, and each individual project will contribute helpful information for setting up such programs relevant to the setting in which they occur.

Current newborn screening programs tend to vary globally both in the number of conditions included on the screen and screening practice in general. In Europe, for example, ~ 85%-100% of the 4.2 million babies born each year receive some form of screening with a range of 2-40 or more disorders on the screen. In the US, nearly 100% of the estimated 3.7 million babies born each year receive NBS, which includes 35 core conditions and 26 secondary conditions.

The clinical utility of NGS-based testing in neonates with indications of genetic disease is well established. Clinical studies such as NSIGHT1 and Project Baby Bear have demonstrated that when used as a first-line test, GS reduces healthcare expenditures by $6,000 to 15,000 per child and between $1 M to $3 M per health

system[6,8,9] and can be cost-neutral or cost-saving[35]. Thus, it is reasonable to suggest that early identification of treatable conditions with NGS-based NBS will also have long-term and potentially cost-saving impacts.

A rapid turnaround time from sampling to report is not a priority for most respondents, who would rather gradually decrease the time-to-result while avoiding compromising more essential aspects such as quality control and confirmatory testing. However, if the long-term goal is to implement NBS that is timely enough for effective intervention, turnaround time is an important component as well as minimal disruption to current NBS programs.

Besides technical feasibility, several challenges linked to NGS implementation in a screening and public health program are shared between countries and initiatives. Those highlighted by survey responses include the development of accessible clinical care pathways for all screened diseases, ethical challenges related to autonomy, information and consent, long-term storage of genomic data, and integration or linkage to medical records. While a discussion of legal, ethical, and privacy concerns is critical when considering the use of genomic information in NBS programs, they were out of scope for the present study which was primarily focused on providing an assessment of planned and ongoing NGS-based NBS programs in Europe.

The survey also revealed an interest in engaging with relevant stakeholders and a recognition that engagement, awareness, and education are necessary components of implementation. However, plans for these activities were not well developed in all studies. Building the capacity of the workforce including laboratory technicians, specialized physicians, midwives, and nurses with varying degrees of involvement in NBS will be key to meeting the increased demands for clinical services downstream of expanded NGS-based NBS programs. Compromising uptake of current NBS programs by the introduction of genomic testing is a concern shared by many. Fostering public trust through engagement as well as education and information of the public are key elements to ensure that uptake of current NBS programs will not be compromised by the introduction of genomic screening tests[36]. The development of preference studies to better understand conditions for the acceptability of genomic screening will help inform an optimal implementation of novel technologies alongside traditional and existing NBS programs.

In conclusion, there are many initiatives being developed in Europe that will explore the utility and feasibility of NGS approaches in NBS programs. This descriptive survey of current programs ongoing or in planning across Europe is an opportunity to survey the landscape, share knowledge and experiences, and reflect on the path towards future implementation. While the projects are heterogeneous in design and maturity, each has the opportunity to contribute information that will enable responsible implementation of NGS in NBS, helping to identify what additional evidence is needed for adoption and informing future research. Confirmatory testing, follow-up protocols of the newborns, conditions for public acceptability, and tracking of downstream healthcare costs are all elements that would benefit from a more unified approach across initiatives. Considering the low prevalence of rare diseases and the small datasets generated by current pilots, sharing data across initiatives will be critical to provide sufficient evidence to demonstrate the clinical utility and cost-effectiveness of NGS in NBS and to consider future implementation within the national healthcare systems and public health programs. We hope that this overview of European NGS-based NBS initiatives will encourage communication and collaboration across countries, in Europe and beyond, avoiding duplication of effort, identifying priorities for resource allocation, and leading to consensus messaging for the expansion of NBS programs around the world.

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Authors' contributions

Involved in the planning and developing of the main conceptual ideas: Bros-Facer V, Patch C
Developed the survey with input from Maria Martinez-Fresno: Bros-Facer V, Taylor S
Analyzed the results of the survey: Bros-Facer V
Contributed to the writing of the manuscript: Bros-Facer V, Taylor S, Patch C

Availability of data and materials

Individual responses to the survey are confidential data that will be destroyed upon acceptance of the manuscript for publication.

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Conflicts of interest

Bros-Facer V and Taylor S are employees of Illumina, Inc. Patch C has no conflicts of interest to declare.

Ethical approval and consent to participate

Not applicable.

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Not applicable.

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Opinion

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Analysis of genomics implementation in newborn screening for inherited metabolic disorders: an IRDiRC initiative

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Abstract

Since its inception in 1963, newborn screening (NBS) has played a pivotal role in early detection and the establishment of appropriate care for infants and children afflicted with inherited metabolic disorders (IMDs). Despite significant advancements in biomarker identification and metabolomics, current NBS protocols only cover a fraction of known IMDs. The integration of genomics holds promise for expanding the scope of standard NBS, albeit presenting additional challenges. Drawing from the experiences of the authors across three European countries, this article reviews the current landscape of conventional NBS for IMDs and explores the potential integration of genomic tools as a primary screening tier. Recommendations are provided for the seamless transition to genomic NBS, considering factors such as regional birth prevalence differentials, treatability of conditions, and technological capabilities.

Keywords: Newborn screening, inborn errors of metabolism, inherited metabolic disorders, rare diseases, biomarkers, genomics, genome sequencing

INTRODUCTION

The International Rare Diseases Research Consortium (IRDiRC) is a global collaborative initiative on rare diseases research launched in 2011 by the European Commission (EU) and the United States of America National Institutes of Health (NIH) to accomplish the vision to enable all people living with a rare disease to receive an accurate diagnosis, care, and available therapy within one year of coming to medical attention. The Newborn Screening (NBS) Initiative was launched in June 2022 to tackle the increasing interest in NBS for identifying rare diseases at an early stage, but also to highlight the challenges that exist in implementing the screening programs across different regions of the world. While next-generation sequencing (NGS) offers the opportunity to screen for genetic disorders through a single test, there are still challenges in terms of overall cost and accessibility to complex and appropriate equipment.

The remarkable advancements in NGS technology have facilitated the discovery of novel inherited metabolic disorders (IMDs) or inborn errors of metabolism, irrespective of the presence of conventional blood, urine, or cerebrospinal fluid markers. Indeed, the latest International Classification of IMDs (ICIMD) aims to include any primary genetic condition in which alteration of a biochemical pathway is intrinsic to specific biochemical, clinical, and pathophysiological features, regardless of whether laboratory biochemical tests are currently available[1,2]. Rare genetic diseases may go unrecognized for weeks, months, or even years until clinical manifestations are evident, and often when it is too late to establish an adequate outcome with optimal treatment[3]. However, the increasing use of techniques such as metabolomics with tandem mass spectrometry (MS/MS)[4] and NGS has expanded the possibilities of diagnosing patients[5,6]. Still, the complex clinical picture of IMDs, in combination with their rarity, makes the early clinical recognition of these rare conditions challenging[7,8].

The establishment of an IMD diagnosis is generally supported by clinical suspicion and biochemical investigations. Currently, NGS technology has grown as an essential tool for rapid and effective diagnostics even prior to complex functional studies (i.e., enzyme activities). Targeted NGS approaches are currently being implemented in clinical practice, and a clinical exome strategy has facilitated the simultaneous assessment of different IMD phenotypes and the study of undiagnosed clinical problems for which a genetic disease is considered[9,10].

NBS represents a vital public health preventative intervention that allows the early diagnosis of a broad spectrum of genetic diseases. The primary goal of NBS programs is to screen for genetic diseases with the purpose of promptly diagnosing pediatric diseases for which specific effective therapeutic interventions are available. For those IMDs with available treatment (e.g., nutritional, pharmacologic, organ transplant, genetic), pre-symptomatic identification is very beneficial, and this is the main reason for newborn screening. NBS, based on metabolic biomarkers, was initially started with a single amino acid, phenylalanine, for phenylketonuria[11,12]. The resounding success of this first approach resulted in a rapid expansion of the biochemical NBS in dried blood spots (DBS), which has been increasingly introduced in the last few years in many countries as a public health program[13,14]. However, there is a group of IMDs without reliable biochemical markers in DBS (e.g., some urea cycle disorders or citrin deficiency), while other IMDs may need different methods than MS/MS (e.g., galactosemia, biotinidase deficiency), such as

enzyme activities[15]. For the screening of some diseases such as GALT deficiency and lysosomal disorders, the enzyme activities may be measured by MS/MS, but the workflow must be adapted to the individual NBS center[16]. Moreover, in some disorders such as cystinuria and orotic aciduria, the specific biomarker is more accurately analyzed in urine samples, sometimes used to complement DBS. This is further complicated in X-linked conditions, such as Fabry disease, Hunter syndrome, and X-linked adrenoleukodystrophy[17], in which the differentiation between an affected female carrier and a healthy individual may be challenging.

Adding a new disease that needs additional methodology to a national or regional NBS program not only increases cost compared to using current methods, but also uses more of the already limited material of the DBS sample. The actual NBS panorama in Europe is very heterogeneous[18], varying from country to country and even within the same country, such as the case of Spain, where a minimum core of diseases to screen is imposed by the Ministry of Health of the Central Government[19-21]. Still, the 17 autonomous regions, with 20 NBS centers, are free to develop further local implementations of NBS. This situation brings a problem of inequity where, depending on the place of birth, one can have, for example, a positive detection of a fatty acid oxidation disorder or not. Of course, there are justified reasons for the NBS heterogeneity among different European regions depending on the varied frequency of some conditions, for example, in northern countries compared to the Mediterranean ones, but at the same time, it may give rise to inequity of care and may present difficulties with immigrants where it might not always be clear what NBS has been done in a neonate[22-25].

Although conventional NBS is a successful program, it has several limitations, some resulting from the lack of a reliable neonatal biochemical marker, such as Wilson disease, while others being of organizational/administrative, economic, or methodological reasons (e.g., homocysteine for classical homocystinuria is not commonly measured as a first tier despite being the best marker). The goal of any NBS program is to deliver rapid results to enable the initiation of a timely therapeutic strategy that would avoid the appearance of irreversible disease complications. The availability of a rapid NBS result, indeed, depends on various organizational factors, such as the established hours of life for the sample collection, the model of transport to the reference NBS laboratory, and the coordination in the reference center until management by the clinical metabolic team of the newborn tested positive is fully in place. The conventional NBS for metabolic diseases, mainly of the intermediary metabolism (amino acids, organic acids, carbohydrates, and fatty acids), was considered to require that the newborn had taken some food for at least 24 h to avoid false negatives, but this consideration has been found not to be true for every biomarker (especially if ratios are being used)[26,27]. Other known limitations of conventional NBS include preterm infants, parenteral nutrition, transfusions, or metabolic decompensation due to various causes. In some centers, the collection of DBS samples is performed in two steps, across two different days, and even including the collection of a dried urine sample[28].

Since IMDs, by definition, have a genetic origin, there is an ongoing discussion to add other treatable IMDs into NBS, using NGS as the screening test. Genomic testing as a first tier for IMDs has already been introduced in the clinical setting for the rapid diagnosis of severe pediatric conditions in neonates and older children in intensive care units[29], and some pilot studies are underway for the use of genome sequencing techniques for NBS (gNBS)[30-32]. While gNBS may provide a more extensive disease identification[33], and independence from the blood collection timing, there are also inherent specific challenges and controversies, encompassing technical, interpretative, social, ethical, and economic aspects, as well as implications at the healthcare level[34]. Although well known in a diagnostic context (symptomatic newborns), the analytical and clinical validity, sensitivity, and specificity of genome sequencing have not been extensively examined in a screening context, mainly concerning healthy newborns[35]. Furthermore,

even in a well-defined context of conditions selected for the screening, there is the possibility of identifying incidental findings, late-onset conditions, and non-treatable diseases, thereby affecting individuals' autonomy in deciding whether to be informed or in self-determining their future life. In addition, the ownership, custody, and protection of genetic data are still a matter of controversy. Altogether, it raises ethical questions that are difficult to address[8]. All these and other open questions must be carefully pondered before national genomic screening programs are implemented. The most prudent approach to implement, particularly when considering cost containment while evaluating analytical and clinical validity, is using targeted gene panels that screen for treatable conditions[36,37].

This report presents an analysis of the present situation of NBS for IMDs in parts of Europe, including a potential set of treatable IMDs not screened today for the reasons described before. Moreover, this report describes the various NGS approaches, such as targeted gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS) with virtual panels, the challenges of including such methods in NBS, and the possible solutions. For practical reasons, we consider WES and WGS, such as "genome sequencing with short reads" (GS), because the concerns regarding these two comprehensive techniques are, to a large degree, similar.

The current situation with conventional biomarker-based NBS (BIO-NBS)

NBS is a public health program that includes a multidisciplinary organization responsible for overseeing the entire screening process, from taking the sample to diagnostic confirmation and referral to appropriate clinical care pathways, improving the quality of the total process based on short and long-term data. Together, this requires a tuned coordination of a flexible and sustainable entire NBS system.

Table 1 lists the conditions included in the conventional NBS programs of three European countries, Spain, Netherlands, and Italy, based on the authors' expertise. As anticipated, the emerging panorama is exceptionally heterogeneous for the list of diseases tested and for technical aspects, such as timing of blood sampling, quantity of blood, and cut-offs used. For example, the age at which the sample is taken may influence the cut-off levels used to classify a sample as abnormal[18].

The purpose of Table 1 is to provide an inventory of IMDs that have at least one informative biomarker to be screened in a population program. Most of these diseases are concurrently screened using tandem MS, referred to as expanded newborn screening. The informative biomarkers commonly used for each disease, along with the second-tier tests that are useful to improve the specificity of the initial screening and the appropriate confirmation methods, are extensively described in the literature and clinical guidelines[26].

In Spain, the official recommendation is to obtain the DBS sample at 24-72 h of life and transport the sample to the NBS laboratory at 3-4 days; the lab result should be ready in < 4 days, so the result of a first sample should be optimally ready in < 10 days after the obtention of the DBS sample, and < 20 days in case of inconclusive results and a second sample is needed for verification[20].

In Netherlands, the time of blood sampling varies from 72 to 168 h after birth, usually after 96 h in combination with the hearing test. Samples are afterward dispatched via regular post to 5 NBS laboratories. For most IMDs, except MMA, PA, MPS1, and ALD, the sample from the child should arrive at the metabolic clinic by day 10 at most.

In Italy, DBS samples are collected at 48-72 h of life and sent to the regional NBS laboratory for the first-tier test via a dedicated transport service that ensures delivery of the samples within 24/48 h of collection and,

Table 1. Different numbers of IMDs included in the conventional NBS in three selected European countries (Spain, Netherlands, and Italy)

Condition	Gene/s	Treatments approved by the European Medicines Agency (not necessarily implying that the treatment is available in a specific country)	NBS-(Andalucía/ Spain)	NBS-Netherlands	NBS-Italy
Biotinidase deficiency	*BTD*	Biotin		X	X
Multiple carboxylase (MCD) Holocarboxylase synthetase deficiency	*HLCS*	Biotin	X	X	X
Classic galactosemia (GALT) deficiency	*GALT*	Galactose free diet		X	X
Galactokinase (GALK) deficiency	*GALK1*	Galactose/lactose-restricted diet		X	
Hyperphenylalaninemia (HPA)	*PAH*	Low phenylalanine diet, tetrahydrobiopterin	X	X	X
Phenylketonuria (PKU)	*PAH*	low phenylalanine diet, pegvaliase, tetrahydrobiopterin	X	X	X
Tetrahydrobiopterin (BH4) deficiency	*GCH1*, *PCBD1*, *PTS*	Tetrahydrobiopterin, levodopa combined with a decarboxylase inhibitor, 5-hydroxytryptophan	X	Tested only if phenylalanine is increased	X
Dihydropterin reductase (DHPR) deficiency	*QDPR*	Tetrahydrobiopterin, levodopa combined with a decarboxylase inhibitor, 5-hydroxytryptophan, low phenylalanine diet	X	X	X
Maple syrup urine disease (MSUD)	*BCKDHA*, *BCKDHB*, *DBT*	Dietary management, thiamine	X	X	X
Branched-chain ketoacid dehydrogenase kinase (BCKDK) deficiency	*BCKDK*	Dietary management, branch chain amino acid supplementation	X		
Tyrosinemia type 1	*FAH*	Nitisinone, low-phenylalanine-tyrosine diet	X	X	X
Tyrosinemia type 2, and type 3	*TAT*	Low-phenylalanine-tyrosine diet	X		X
Argininemia deficiency (ARG1D)	*ARG1*	Protein restriction, liver transplant, sodium benzoate, phenylbutyrate (sodium, glycerol)	X		X
Argininosuccinic aciduria (ASA)	*ASL*	Protein restriction, liver transplant, sodium benzoate, phenylbutyrate (sodium, glycerol)	X		X
Citrullinemia type 1	*ASS1*	Protein restriction, sodium phenylbutyrate, glycerol phenylbutyrate, L-carnitine, liver transplantation	X		X
Citrullinemia type 2	*SLC25A13*	MCT milk and lactose-free milk, lipid-soluble vitamins, and ursodeoxycholic acid. Liver transplantation	X		X
Methionine adenosyltransferase (MAT) deficiency	*MAT1A*	Low methionine diet (some patients)	X		X
Classic Homocystinuria (CBS) deficiency	*CBS*	Vitamin B6 (pyridoxine), methionine-restricted diet, folate, vitamin B12, betaine	X		X
Remethylation defects	*MTHFR*	Betaine, 5-methyltetrahydrofolate	X		X
Glutaric aciduria type 1 (GA1)	*GCDH*	avoid fasting, carnitine, protein-restricted diet, restrict lysine, hydroxylysine, and tryptophan	X	X	X
Isovaleric acidemia (IVA)	*IVD*	Low protein diet, l-carnitine, glycine	X	X	X
3-methylglutaconic acidemia (primary 3-MGA)	*AUH*, *HMGCL*, *CLPB*, *SERAC1*	Low-protein diet, carnitine	X		X
2-methyl butyryl glycinuria (SBCAD)	*ACADSB*	Carnitine (avoidance of valproate)	X		
Methylmalonic acidemia (Mutase deficiency, CblA, CblB)	*MMUT*, *MMAA*, *MMAB*	IM hydroxycobalamin, carnitine, diet, N-carbamylglutamate, liver transplant	X	Intended to find only MMUT	X
Methylmalonic acidemia with homocystinuria (CblC, CblD)	*MMADHC*, *MMACHC*	IM hydroxycobalamin, carnitine, diet, betaine, N-carbamylglutamate, liver transplant	X		X
3-OH-3-methylglutaryl-CoA lyase deficiency (HMG)	*HMGCL*	IV glucose during acute episodes, avoid fasting, carnitine, protein-restricted diet	X	X	X
Beta-ketothiolase deficiency (BKT) Mitochondrial acetoacetyl CoA	*ACAT1*	Avoid fasting, carnitine, riboflavin, protein-restricted diet	X		X

thiolase					
3-methyl crotonyl CoA carboxylase (MCC)	*MCCC1/MCC2*	IV glucose during acute episodes, avoid fasting, carnitine, protein-restricted diet	X	X	X
Propionic acidemia (PA)	*PCCA, PCCB*	Diet, carnitine, biotin, metronidazole, liver transplantation, N-carbamylglutamate	X	X	X
MCAD medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	*ACADM*	Avoid fasting	X	X	X
VLCAD very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	*ACADVL*	Avoid fasting, carnitine, restrict LCFA, bezafibrate, triheptanoin	X	X	X
long-chain 3-OH acyl-CoA dehydrogenase deficiency (LCHAD) mitochondrial trifunctional protein (TFP) deficiency	*HADHA, HADHB*	IV glucose during acute episodes, avoid fasting, carnitine, restrict LCFA, bezafibrate, MCT, triheptanoin	X	X	X
carnitine palmitoyl-1 transferase (CPT-1) deficiency	*CPT1A*	Avoid fasting, low-fat and high-carbohydrate diet, MCT, triheptanoin	X	X	X
carnitine palmitoyl-2 transferase (CPT-2) deficiency	*CPT2*	Bezafibrate, high-carbohydrate and low-fat diet, carnitine, MCT, triheptanoin	X		X
carnitine-acylcarnitine translocase (CACT) deficiency	*SLC25A20*	High-carbohydrate and low-fat diet, MCT, frequent feeds, carnitine, avoid fasting, triheptanoin	X		X
short-chain acyl-CoA dehydrogenase (SCAD) deficiency	*ACADS*	As most individuals are asymptomatic, there is no need for treatment but careful follow-up	X		X
Isobutyrylglycinuria	*ACAD8*	As most individuals are asymptomatic, there is no need for treatment but careful follow-up	X		
multiple acyl-CoA dehydrogenase deficiency (MADD)	*ETFDH*	Riboflavin, carnitine, glycine, Coenzyme Q10 supplementation, fat restriction, avoidance of fasting, and a diet rich in carbohydrates	X		X
CUD/CTD (carnitine uptake/carnitine transporter deficiency)	*SLC25A20*	High-carbohydrate and low-fat diet, medium chain triglycerides, frequent feeds, carnitine, avoid fasting, triheptanoin	X		X
X-linked Adrenoleukodystrophy (X-ALD)	*ABCD1*	HSCT, BMT, gene therapy (elivaldogene)	Pilot	X	Pilot
Cystic Fibrosis (CF)	*CFTR*	Ivacaftor, tezacaftor, lumacaftor, pancreatic enzyme, inhaled antibiotics, dornase alfa	X	X	X
Mucopolysaccharidosis type 1 (MPS1)	*IDUA*	ERT: laronidase, BMT, HSCT		X	Regional
Pompe disease (GSD II)	*GAA*	ERTs: avalglucosidase alfa and alglucosidase alfa			Regional
Fabry disease	*GLA*	ERTs: agalsidase alfa, agalsidase beta, pegunigalsidase alpha PCT: migalastat			Regional
Gaucher disease	*GBA*	ERTs: imiglucerase; velaglucerase alfa; and taliglucerase alfa. SRT: miglustat, eliglustat. PCT: Ambroxol			Regional
Metachromatic leukodystrophy (MLD)	*ARSA*	HSCT - BMT, atidarsagene autotemcel (Libmeldy)			Regional

NBS in Spain is very heterogeneous among the autonomous communities and cities. In this table, the case of Andalucía, one of the most populated regions of Spain and the site where one of the authors (RY) is responsible for the NBS, is presented as representative of the different communities of Spain. X: condition included. For Italy, X means performed in all regions of the country. Regional: only in some regions of the country. Pilot: still under evaluation. National NBS in Italy includes 48 conditions by law; national NBS in Spain includes a minimum core of 7 diseases, but a variable expanded NBS is in place in most of the regions, such as Andalucía (35 conditions). In Netherlands, the NBS screening program is standardized across the whole country. ERT: Enzyme replacement therapy; SRT: substrate reduction therapy; PCT: pharmacological chaperone therapy; BMT: bone marrow transplant; HSCT: hematopoietic stem cell transplantation; MCT: medium-chain triglycerides; LCFA: long-chain fatty acids; IV: intravenous; IM: intramuscular.

only in exceptional circumstances, within 72 h, while in other countries, the regular post or regular post with extra care and velocity, called medical post, is being used.

In general, the reference NBS laboratory carries out the first-tier test mainly using the MS/MS method. Minimizing the number of "false positives", i.e., those subjects who are shown positive for the screening test but who are not ill, is relevant in terms of containing healthcare costs and reducing the social impact of a positive test that can have emotional implications for parents. For this purpose, second-tier tests have been developed, always carried out on the same DBS and with a higher specificity, for an initial evaluation of the first screening tests, either positive or negative. Usually, when a result is obtained, a re-test is performed with the same sample to establish its reference range, and if there is divergence, the same test is repeated using another DBS of the same screening sample. The selected second tier test may be performed within the rest of DBS from the original screening test (i.e., amino acids, succinylacetone, total homocysteine, genomic sequencing), or it may be necessary to collect urine (i.e., organic acids, orotic acid, acylglycines) sometimes with a confirmatory second screening sample. Most of the time, the referral is organized after the re-test or the triplicate sample without waiting for the result of the second tier. However, in some cases of vitamin B12 deficiency, the increased level of C3 may not be sufficiently discriminative and it might be wise to wait for the second tier for MMA/PA before referral takes place. In some countries, genetic diagnostic confirmation tests are performed after the second tier or as an alternative to these for disorders such as fatty acid beta-oxidation defects and some organic acidurias. The blood spot is taken from all live births, including live births with subsequent death between 48 and 72 h of life, for which the sampling is carried out peri-mortem, communicating this circumstance to the regional NBS laboratory.

As already described in the introduction, a wide range of IMDs cannot or cannot easily be identified due to a lack of clear biochemical markers in the DBS. Table 2 presents examples of treatable IMDs without a sufficiently robust biochemical marker where NGS is used as a confirmatory test or where NGS as first-tier could be the best option for the screening. The number of diseases in this category heavily depends on several factors, and the complete list is over the scope of this report. Indeed, the list of IMDs beyond the Wilson & Jungner criteria (1968) is influenced by the discussion on treatability versus actionability of the disease, knowledge on the natural course of the disease, ease to differentiate patients presenting early from individuals that may have a later presentation, in which sometimes the definition of "later presentation" is unclear (e.g., presentation after one year, ten years, or as late as adulthood), and also by several other factors.

Present situation with NGS as a first-tier test in NBS

A wide range of pilot studies based on NGS are ongoing or planned around the world to demonstrate the technical feasibility of NGS as a first-tier test for NBS[27,32]. The majority of these studies addresses only a single or a few aspects related to the use of genomic methodologies in NBS, such as technical, interpretative, social, ethical, and economic challenges, to name a few[38,39].

If NGS will be used as the first tier, there is a need to build a database with the NGS data and their biomarkers -if performed - and the conclusion of whether it is a 4-5 genetic variant, or still a VUS or (likely) benign. This will require solutions for long-term storage of data. At present, biomarker-based NBS (Bio-NBS) samples are stored for a minimum of 5 years (following established European ISO regulations). They can be revised in various situations, e.g., when looking for causes of an unexpected death in childhood. In this case, parents or the individuals themselves (if the related data or collected samples are stored for patients older than 16 years of age) should give their consent for data sharing and storage. This process would encourage the construction of large data libraries with NGS information but should be limited to the genetic diseases agreed on before, and it must comply with the bio-bank storage regulations and informed consent specifications for genomic samples.

Table 2. IMDs without a sufficiently robust biochemical marker where NGS may be used as a first-tier test

Condition	Gene/s	Treatment
N-acetylglutamate synthase, NAGS deficiency	*NAGS*	N-carbamylglutamate
Carbamoyl-phosphate synthetase I, CPS1 deficiency	*CPS1*	Protein restriction, citrulline, sodium benzoate, phenylbutyrate, liver transplantation, N-carbamylglutamate
Ornithine transcarbamylase, OTC deficiency	*OTC*	Protein restriction, citrulline, sodium benzoate, phenylbutyrate, glycerol phenylbutyrate, liver transplantation
Carbonic anhydrase VA, CAVA deficiency	*CA5A*	N-carbamylglutamate, limit protein with illness, ensure caloric intake
Delta-1-pyrroline-5-carboxylate dehydrogenase deficiency, Hyperprolinemia type II	*ALDH4A1*	Pyridoxine
Mitochondrial Ornithine transporter 1 (ORNT1) deficiency, HHH syndrome	*SLC25A15*	Low-protein diet, citrulline, arginine
Mitochondrial aspartate glutamate carrier 2, Citrin deficiency	*SLC25A13*	MCT milk and lactose-free milk, lipid-soluble vitamins, and ursodeoxycholic acid. Liver transplantation
Lysinuric protein intolerance (LPI)	*SLC7A7*	Citrulline
Hyperinsulinism-hyperammonemia syndrome	*GLUD1*	Diazoxide, somatostatin analogs, nifedipine, glucagon, IGF-1, glucocorticoids, growth hormone, pancreatic resection, mTOR inhibitors, GLP-1 receptor antagonists, sirolimus
Thiamine transporter 1	*SLC19A2*	Thiamine (vitamin B1), insulin
Biotin-thiamine responsive basal ganglia disease (thiamine transporter 2)	*SLC19A3*	Thiamine (vitamin B1) and biotin
Thiamine metabolism dysfunction syndrome 5	*TPK1*	Thiamine (vitamin B1)
Transcobalamin II deficiency	*TCN2*	Hydroxocobalamin
Brown-Vialetto-van Laere syndrome 1, 2 (Riboflavin transporter deficiency)	*SLC52A3* *SLC52A2*	Riboflavin (vitamin B2)
Manganese transporters	*SLC30A10*, *SLC39A14*	Manganese chelation therapy with EDTA-CaNa2
Magnesium transporters	*SLC41A1*, **ABCC6*, ***ABCG5/ABCG8*	*Etidronate, anti-hypertensive, calcitriol and oral phosphate supplements **Diet low in shellfish sterols and plant sterols, ezetimibe, cholestyramine
Epithelial magnesium transporter deficiency, Hypomagnesemia with secondary hypocalcemia	*TRPM6*	Magnesium
Hypomagnesemia-hypercalciuria-nephrocalcinosis	*CLDN16*, *CLDN19*	Magnesium, thiazide, renal transplant
Bartter syndrome type 1, 2	*SLC12A1*, *KCNJ1*	Sodium chloride, potassium chloride, indomethacin
Calcium transporters and receptors: Autosomal dominant hypocalcemia* Neonatal hyperparathyroidism and familial hypocalciuric hypercalcemia type I**	*SLC25A24*, *SLC25A13*, *SLC8A1*, *SLC8B1*, *SLC30A10*, *SLC24A2*, *CASR*	Thiazide diuretics, calcium, calcitriol* Bisphosphonate, parathyroidectomy, cinacalcet**
Hyperoxaluria type 1 (hepato-renal), type II, type III	*AGXT*, *GRHPR*, *HOGA1*	Lumasiran, pyridoxine, drinking large volumes, alkalinization of urine, pyrophosphate-containing solutions, liver-kidney transplant
Congenital Defects of Glycosylation (CDG)	*ALG1-CDG*, *MPI-CDG*, *PMM2-CDG*, *PGM1-CDG*	Mannose, D-galactose, fucose
mtDNA disorders: Thymidine kinase deficiency	*TK2*	Deoxycytidine (dC) and deoxythymidine (dT)
Molybdenum cofactor deficiency	*MOCS1*, *MOCS2*, *GEPH*	Cyclic pyranopterin monophosphate (*MOCS1*)
Pyrimidine disorders: orotic aciduria, early infantile epileptic encephalopathy-50	*UMPS*, *CAD*	Uridine, triacetyluridine
Wilson disease	*ATP7B*	Zinc, D-penicillamine, trientine
Menkes disease	*ATP7A*	Subcutaneous injections of copper histidine or copper chloride
Cerebral creatine deficiency syndrome 2	*GAMT*	Creatine monohydrate and ornithine supplementation. Arginine restriction
Cerebral creatine deficiency syndrome 3	*GATM*	Creatine monohydrate
RPE65 associated Leber congenital amaurosis, early-onset severe retinal dystrophy	*RPE65*	Gene therapy (Luxturna)

Glut-1 deficiency	*SLC2A1*	Ketogenic diet
Cystinosis (Infantile Nephropathic)	*CTNS*	Cysteamine, Cysteamine slow release, cysteamine eye drops, Carnitine, GH, vit D, phosphate, citrate, kidney transplant
Mucopolysaccharidosis type 1 (MPS1)[#]	*IDUA*[#]	Laronidase ERT, BMT, HSCT[#]
Mucopolysaccharidosis type 2 (MPS2)[#]	*IDS*[#]	Idursulfase ERT, BMT, HSCT[#]
Mucopolysaccharidosis type IVA[#]	*GALNS*[#]	Elosulfase alpha ERT, HSCT[#]
Mucopolysaccharidosis type VI[#]	*ARSB*[#]	Galsulfase ERT, HSCT[#]
Mucopolysaccharidosis type VII[#]	*GUSB*[#]	Mepsevii ERT, HSCT[#]
Pompe disease (GSD type 2)[#]	*GAA*[#]	Avalglucosidase alfa and alglucosidase alfa ERT[#]
Fabry disease[#]	*GLA*[#]	Agalsidase alfa and beta ERT, pegunigalsidase ERT, migalastat PCT[#]
Gaucher disease[#]	*GBA*[#]	ERT: imiglucerase; velaglucerase alfa; and taliglucerase alfa; SRT: miglustat, eliglustat; PCT: Ambroxol[#]
Acid sphingomyelinase deficiency -ASMD1[#]	*SMPD1*[#]	Recombinant human acid sphingomyelinase, ERT: olipudase alfa[#]
Niemann-Pick type C[#]	*NPC1*[#]	SRT: miglustat[#]
Neuronal Ceroid lipofuscinosis (CLN2)[#]	*TPP1*[#]	Cerliponase alfa ERT[#]
Lysosomal Acid lipase deficiency (LALD)[#]	*LIPA*[#]	Sebelipase alfa ERT[#]
Metachromatic leukodystrophy (MLD)[#]	*ARSA*[#]	HSCT, BMT, atidarsagene autotemcel (Libmeldy)[#]
Krabbe disease[#]	*GALC*[#]	HSCT, BMT[#]
Alpha-mannosidosis[#]	*MAN2B1*[#]	Velmanase alfa ERT[#]
AADC deficiency	*DDC*	Pyridoxine/pyridoxal phosphate, folinic acid, dopamine agonists, MAO B inhibitors Gene therapy eladocagene exuparvovec (Intracerebral Upstaza[TM])
PNP deficiency (SCID)	*PNP*	HSCT
ADA deficiency (SCID)	*ADA*	PEG-ADA ERT, HSCT, Gene therapy Strimvelis[TM]
Abetalipoproteinemia	*MTTP*	Vitamin E, A, D
Adenine phosphoribosyltransferase deficiency	*APRT*	Allopurinol
Barth syndrome	*TAZ (Tafazzin)*	Cardiac transplantation, granulocyte colony-stimulating factor (G-CSF), uncooked cornstarch given prior to bedtime, elamipretide
Cerebrotendinous xanthomatosis	*CYP27A1*	Chenodeoxycholic acid (CDCA), cholic acid
Crigler-Najjar syndrome type I	*UGT1A1*	Intensive phototherapy, albumin infusions, and plasma exchanges, liver transplantation, gene therapy (http://clinicaltrials.gov/show/NCT03466463)
Familial chylomicronemia syndrome	*LPL*, *APOC2*, *LMF1*, *APOA5*, *GPIHBP1*	Low-caloric diet, fibrates
Familial hypercholesterolemia	*APOB*, *LDLR*, *PCSK9*	diet, statin, evinacumab, ezetimibe, apheresis, evolocumab, lomitapide
Fructose-1,6-bisphosphatase deficiency	*FBP1*	Restriction of fructose or sucrose, avoid prolonged fasting, IV glucose during metabolic decompensation
Galactokinase deficiency	*GALK1*	Lactose/galactose-restricted diet
Glycogen storage disease type I	*G6PC1* *SLC37A4*	Restriction of fructose or sucrose, avoid prolonged fasting, IV glucose during metabolic decompensation
Glycogen storage disease type III	*AGL*	High-protein diet with corn starch supplementation
Homocystinuria-megaloblastic anemia	*MTTR*, *MTR*	Vitamin B12
Combined immunodeficiency and megaloblastic anemia with or without hyperhomocysteinemia	*MTHFD1*	Hydroxocobalamin, folinic acid, betaine
Primary coenzyme Q10 deficiency	*COQ4*, *COQ6* *COQ2*	Coenzyme Q10
Pyridoxine-dependent epilepsy	*ALDH7A1*	Pyridoxine, lysine-restricted diet, arginine supplementation
Pyridoxamine 5'- phosphate oxidase deficiency	*PNPO*	Pyridoxal 5'-phosphate (PLP) and pyridoxine
Pyridoxal 5'-phosphate binding protein deficiency	*PNPBP*	Pyridoxine (first line) and Pyridoxal 5'-phosphate

X-linked hypophosphatemic rickets	*PHEX*	Phosphate supplementation, active vit D, Burosumab (monoclonal Ab)
Hypophosphatemic rickets with hypercalciuria	*SLC34A3*	Phosphate supplement, active vit D
Hypophosphatasia	*ALPL*	Tissue-nonspecific alkaline phosphatase (TNSALP) ERT - asfotase alfa, avoid bisphosphonates
Congenital serine biosynthesis defects	*PHGDH* *PSAT1* *PSPH*	Serine, glycine
Cerebral folate transport deficiency	*FOLR1*	Folinic acid

This list is not intended to be a comprehensive list of treatable IMDs but just a useful indicator. The degree of evidence of the treatments is variable and may be mutation- or patient-specific. ERT: Enzyme replacement therapy; SRT: substrate reduction therapy; PCT: pharmacological chaperone therapy; BMT: bone marrow transplant; HSCT: hematopoietic stem cell transplantation; MCT: medium-chain triglycerides; IV: intravenous; IM: intramuscular; IGF-I: insulin growth factor-I; GLP-I: glucagon-like peptide-1 receptor; mTOR: mammalian target of rapamycin inhibitors. #indicate list of lysosomal disorders that are presently best screened by MS/MS-based enzyme assay followed by genetic confirmation; */** indicate the relationship of the disease or the gene with the correspondent treatment.

As illustrated in the first part of this review, the analytical and clinical validity, sensitivity, and specificity of genome sequencing have not been extensively examined in a screening context. It is imperative to take into account that the primary beneficiaries of NBS are healthy newborns, thus emphasizing the paramount importance of ensuring the integrity and safety of screening methodologies to safeguard this vulnerable population[40].

Table 3 highlights several practical hurdles that need to be considered and some possibilities to address these challenges.

Two main paths can be envisioned for the evolution and progress of NBS for IMDs: the first could be a progressive and prudent transition, including a consistent period of co-existence and thorough cross-checking of metabolomics and NGS methodologies, from a biochemical profile to genomic confirmation up to therapy, with progressive side-by-side support of conventional NBS and genomics. The traditional Bio-NBS can yield false-positive or false-negative results and is affected by biochemical substrate-level fluctuations. The genomic DNA extracted from dried blood spots can be used for NGS, generating reliable sequencing results, and NGS may function as a second-tier diagnostic test for NBS in samples with abnormal MS/MS results. Most centers use a multigene panel, comprising a library of genes related to the IMDs, for NBS. Genetic testing as the second tier is more or less replacing the present clinical situation toward the screening system. We would like to emphasize that using biochemical and genomic NBS in parallel may increase the sensitivity of the screening and more newborns may be identified, decreasing the number of false positives.

The second path could include genomics as the first-tier test and biochemistry/metabolomics as diagnostic confirmation of the disease before starting treatment. However, gNBS is currently used as the first-tier test only for those disorders not included in the Bio-NBS because of the lack of a reliable biomarker.

The primary objective of NBS is to diagnose pediatric diseases for which effective therapeutic interventions exist, thereby mitigating symptom onset or progression and improving patient prognosis, quality of life, and familial well-being. These interventions aim to avert irreversible damage, including severe physical and cognitive impairments and, in extreme cases, mortality[41-43].

Today, besides endocrine disorders (CH, CAH), hemoglobinopathies, SCID, and Cystic Fibrosis, most NBS programs detect treatable IMDs that are identifiable in the first days of life, mainly with mass

Table 3. Possible concerns (with some possible answers) when introducing NGS as first-tier in NBS

Issue	Biomarker-based NBS (BIO-NBS)	Targeted NGS physical gene panel (tNGS)	WES/WGS (virtual panels of genes)
False negatives	Less and less due to experience with methods +	Will be high if compared to present BIO-NBS, also if compared to WES and WGS. The number of false negatives depends on managing variants of unknown significance (VUS) and the lack of a condition/gene in the panel +++	Will be less high if compared to tNGS but will still be higher (at the start) if compared to present BIO-NBS. The number of false negatives depends on managing VU ++
False positives	Relatively high, especially for some diseases presently included in BIO-NBS Less in NBS labs that perform second-tier testing +/++	Probably fewer if compared to BIO-NBS if reported "only" class 4 and 5 genetic variants +	Depending on the handling of VUS, little if compared to BIO-NBS if reported "only" class 4 and 5 genetic variants, but possibly higher compared to tNGS +/++
Costs	Increasing due to the growing number of diseases included and various methods used +	Higher at this moment if compared to BIO-NBS but decreasing if more diseases are screened for using the same method ++	Still higher if compared to present BIO-NBS, but depending on the number of included genetic diseases, the price per disease or found patients will decrease, as the costs of the method would not be that much different if the number of diseases increases +++/++++
Time to result or turnaround time	For IMDs usually very short (1-2 days after DBS reaches the NBS lab +	Additional 4 days if compared to BIO-NBS ++	Still an additional 4 days if compared to BIO-NBS. When long reads are included, time will decrease to 2-3 days +/++
Need for big data infrastructure	Not that large +	Probably comparable to BIO-NBS +/++	WES: Clearly larger if compared to BIO-NBS and tNGS WGS: Much larger compared to BIO-NBS, tNGS and WES +++/++++

The arguments in the table represent an indicative analysis. A prolonged global experience in gNBS as a first-tier test for IMDs is necessary to give strong evidence to the comments and evaluation presented above. A semiquantitative score (+ to ++++) in support to the text was added (+: light, ++: mild, +++: moderate, ++++: strong).

spectroscopy[44,45]. Other rare diseases with a genetic basis without detectable biochemical markers that can be treated if identified early, before symptoms and irreversible damage, such as spinal muscular atrophy (SMA), are already included in some national NBS programs[46].

NBS are large-scale programs at a population level targeting all newborns, the very most of whom are healthy. The diagnostic context is different since the test may be tailored to the individual patient, considering the clinical manifestations, and numerous clinical and instrumental data may be used to support the diagnosis.

At present, genome sequencing is increasingly used in clinics, especially for diagnosing severely symptomatic pediatric patients hospitalized in intensive care, where the benefits deriving from achieving a timely diagnosis, including the initiation of specific therapies or appropriate clinical management, balance the costs of the test, still high although decreasing steadily[29]. This application of genome sequencing in severely symptomatic newborns for early and timely diagnosis, and the rapid turnaround time of the test has opened the possibility of using these technologies outside of the diagnostic setting in a screening context as a prevention tool. The availability of such powerful genomic tools may shift the concept of "treatability" underlying NBS toward a broader and sometimes more ambiguous concept of "actionability"[47]. This concept introduces some ethical considerations. Screening for diseases that do not have a treatment with

proven efficacy may still bring eventual benefits for patients and their families, for example, decreasing the time to diagnosis and avoiding disease complications. On the other hand, identifying newborns and infants bearing a late-onset condition and predicting a probable later disease manifestation may cause parental anxiety and stigmatize the affected child, but in spite of that, genetic information may help make decisions for the future. The balance between possible future benefits and the psychological hurdle is difficult to achieve[48].

Another aspect to consider is that currently, the interpretation of genetic variants is supported by the deep phenotyping of patients, which is necessary before performing the test, and through reverse phenotyping after the completion of the genomic test, which helps to evaluate the clinical significance of the variants. This essential exchange process between clinic and laboratory, along with the simultaneous analysis of parents (trio), helps to reduce the uncertainties of genomic results (class 3 variant or VUS)[49]. The lack of phenotype in asymptomatic newborns (screening context) makes this exchange process between laboratory and clinic impossible, allowing for reporting only variants with a high probability of pathogenicity (class 4 and 5), increasing the possibility of false negatives in genomic screening. The database will need to be filled with class 4 and 5 variants, but class 3 (VUS) can also be used if they are being controlled with biomarkers (if possible), to help to know whether it can be judged as a class 4 or 5 variant in the future.

This would possibly create confusion even among expert operators in the field, but we want to emphasize the critical importance of careful variant curation, ensuring that only combinations of biallelic variants that are known to be associated with early-onset disease are reported, and that communicating uncertainties or making predictions of a remote future is not necessarily beneficial.

The use of genomic techniques in this target population brings up unprecedented ethical, psychological, and social issues in the field of screening. All these and other open questions must be carefully considered before national genomic screening programs are implemented[30,39,50].

CONCLUSIONS

Recommendations include assessing the accuracy and predictive capacity of gNBS, by cautiously implementing gNBS with a focus on treatable disorders, utilizing second-tier analyses or biomarkers to refine diagnosis and treatment decisions, and establishing protocols for the close monitoring of conditions with uncertain prognostic implications.

A prudent approach would be to start gNBS by including only a combination of (likely) pathogenic DNA variants associated with an established list of treatable disorders. With this approach, some patients will be missed, but it will limit many false positives. Along the way, we will learn how to reclassify the variants from the missed patients who will manifest these disorders.

The evaluation of early-initiated therapies is imperative for determining treatment efficacy, aligning with the criterion of treatability in NBS inclusion criteria. Indeed, one of the classical criteria for including a condition in the NBS is its present treatability, if possible, with strong evidence, to significantly improve the natural history of the disease for most of the patients bearing such genetic variants.

For those conditions with an expected late-onset manifestation or genetic variants without a precise prediction of the disease evolution, we need to carefully discuss whether these should be included and, if included, protocols for a close follow-up of these newborns must be established with the objective of not missing the possibility of initiating a specific treatment before the appearance of irreversible organ/system damage.

Special consideration is necessary for IMDs with a high risk of presenting very early acute symptoms (organic acidurias, urea cycle disorders), even after a few hours of life, so are the sick newborns during the first 3-5 days of life, as they will need a careful clinical evaluation using rapid diagnostics including present metabolic investigations as well as rapid metabolomics and genome sequencing, if possible in a combined effort.

The introduction of gNBS warrants a judicious and phased approach, centralized in experienced reference centers, and characterized by selective gene panels to ensure harmonization and protocolized utilization. Collaborative efforts, including global data sharing, are essential for optimizing screening outcomes and refining screening protocols over time.

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Pintos-Morell G and van Karnebeek CDM are members of the Diagnostics Scientific Committee of IRDiRC. Tataru EA works at *IRDiRC Scientific Secretariat.* While the other authors have declared that they have no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

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Not applicable.

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Perspective

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Newborn screening in Mexico and Latin America: present and future

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Abstract

The first newborn screening (NBS) program to be implemented in Latin America was in Mexico in 1974, eleven years after the initial NBS programs in other parts of the world. In the last 50 years, progress has been made in implementing and expanding NBS in Mexico and across Latin America, yet children across the region do not fully benefit from this effective public health strategy. Here, we review the progress in the implementation of expanded NBS in Latin America with a focus on Mexico and the challenges faced by its complex healthcare system. In light of new technologies such as genomic sequencing and their potential utilization for NBS, we discuss what the future of NBS may be for Mexico and countries in Latin America and the Caribbean region, given economic and technological constraints.

Keywords: Neonatal screening, NBS, ENBS, gNBS, genetic diseases, early diagnosis, precision health

THE PAST AND PRESENT OF NEWBORN SCREENING IN MEXICO AND LATIN AMERICA

Newborn screening (NBS) is a public health strategy aimed at identifying, in the first days of life, serious conditions that may affect the survival or health of a child, and where early diagnosis and treatment result in improved health outcomes, thus reducing morbidity, disability, and mortality. Although established in 1963 with the first mandated screenings done for phenylketonuria (PKU) in the United States, the implementation of NBS around the world is very heterogeneous. In Latin America, the implementation of NBS programs is unequal and inconsistent. Only 16 of the 33 countries in the region currently have a national NBS program and the number of conditions included varies widely across countries from one condition, namely congenital hypothyroidism (CHT) being screened in 16 countries, up to 29 conditions screened for in Costa Rica [Table 1][1,2]. The development of tandem mass spectrometry (MS/MS) technologies enabled the screening of dozens of metabolites in a single assay, expanding the capabilities of classical biochemical NBS to include disorders of the metabolism of amino acids, organic acidemias, and fatty acid oxidation disorders[3]. Unfortunately, the adoption of MS/MS technologies for expanded newborn screening (ENBS) in Mexico and Latin America is still lagging behind by decades, with only Costa Rica and Uruguay having national ENBS programs in the region[1,2] and a few regional, state, or localized efforts in a handful of countries. Economic conditions, outdated legislation and technology, and limitations on coverage and accessibility have hindered the widespread adoption of NBS in the Latin American and Caribbean (LAC) region. This has resulted in a considerable number of infants with rare diseases going undiagnosed and untreated, resulting in preventable morbidity and mortality in these populations.

In Mexico, NBS was first implemented in 1974, being the first country in Latin America to adopt this public health strategy to screen infants for PKU, CHT, and congenital toxoplasmosis. Due to technical difficulties with the assay, testing for toxoplasmosis was dropped shortly after, whereas initial screening results for PKU and CHT showed the success of the program. In 1977, the NBS program was paused, but reinstated in 1986. In 1988, legislation was approved to make NBS mandatory in medical institutions nationwide for newborns. However, despite the evidence supporting the importance of screening for PKU, this disorder was also removed from the program due to the low number of positive cases detected in the Mexican population, leaving the national NBS program to screen only for CHT[4]. In 1998, the national NBS program was expanded to include PKU, congenital adrenal hyperplasia (CAH), galactosemia (GAL), and biotinidase deficiency (BTD) for all newborns in Mexico. Updated guidelines published in 2002 suggested the expansion of NBS to test for additional disorders based on the recommendations of the National Center of Epidemiological Surveillance (Centro Nacional de Vigilancia Epidemiológica); however, no official mandate was established. New guidelines were published in 2012, emphasizing the importance of the expanded metabolic newborn screening covering at least CHT, CAH, GAL, disorders of amino acid metabolism, disorders of fatty acid metabolism, cystic fibrosis (CF), hemoglobinopathies, severe combined immunodeficiency (SCID), and leaving the possibility open to include other disorders that represent a public health problem. In January 2013, a Congress decree was published, establishing the mandatory implementation of ENBS and ophthalmological screening for all Mexican newborns, as well as retinal and hearing screening for premature newborns, to ensure integral childhood development and the prevention and detection of hereditary and congenital conditions. Despite this, ENBS is still not implemented nationally and the major public healthcare institutions continue to screen for only six conditions: CHT, PKU, CAH, CF, GAL, and glucose-6-phosphate dehydrogenase deficiency (G6PDD)[2,4,5]. Individual institutions and healthcare systems can include additional conditions to screen for based on their budget, their technological and logistical capabilities, and other internal considerations as determined by institutional review committees.

Table 1. Newborn screening programs in Latin American countries

LATAM country	Year first implemented	Current number of conditions included in screening	Public/private access/implementation
México	1974	Screening of 6 to 76 disorders is variable depending on the healthcare system. Main conditions screened for include CHT, PKU, CAH, CF, GAL, BTD, and G6PDD	Public nationwide mandated; public/private ENBS options, a variable number of conditions depending on institution or state
Argentina	1986	Screening for 6 disorders (CHT, PKU, CAH, CF, GAL, BTD)	Public nationwide mandated. Some cities screening for additional disorders
Bolivia	2006	Screening for 4 disorders (CHT, PKU, CAH, and CF). Only CHT is mandated nationwide	Public nationwide mandated but variable per region
Brazil	2001	Screening for 6 conditions (CHT, PKU, CAH, CF, BTD, and Hemoglobinopathies)	Public nationwide. ENBS for metabolic disorders available in some states
Chile	1992	Screening for 2 conditions (CHT and PKU)	Public. ENBS pilot undergoing to expand to 26 conditions
Colombia	2000	Only CHT is screened for nationwide. PKU, CF, GAL, BTD, CAH and Hemoglobinopathies added in 2019 as part of the basic NBS program	Public nationwide mandated; private options available including additional conditions. ENBS pilot program being evaluated to screen for 33 total disorders
Costa Rica	1990	Screening for 29 conditions including CHT, PKU, CAH, CF, GAL, Hemoglobinopathies, MSUD, other amino acid disorders, fatty acid oxidation disorders, and organic acidurias	Public nationwide mandated. Most comprehensive public program in LATAM
Cuba	1986	Screening for 6 disorders (CHT, PKU, CAH, GAL, CF, BTD)	Public nationwide
Ecuador	2011	Screening for 4 disorders (CHT, PKU, CAH, GAL)	Public nationwide
El Salvador	2008	Only screening for CHT	Regional public program
Honduras	2016	Screening for 5 disorders (CHT, PKU, CAH, GAL, CF)	Public with variable coverage
Panamá	2007	Screening for 8 disorders (CHT, PKU, CAH, GAL, CF, Hemoglobinopathies, Sickle cell disease, G6PDD)	Public nationwide mandated
Paraguay	2004	Screening for 3 disorders (CHT, PKU, CF)	Public nationwide
Perú	2012	Screening for 5 disorders (CHT, PKU, GAL, CAH, CF)	Public nationwide
Uruguay	1994	Screening for 28 disorders including CHT, PKU, CAH, CF, Hemoglobinopathies, and additional 23 metabolic conditions including MSUD, other amino acid disorders, fatty acid oxidation disorders, and organic acidurias	Public nationwide Among the most comprehensive public programs in LATAM
Venezuela	1999	Screening for 2 disorders (CHT, PKU)	Public nationwide

NBS: Newborn screening; ENBS: expanded newborn screening; CHT: congenital hypothyroidism; PKU: phenylketonuria; MCADD: medium-chain acyl-CoA dehydrogenase deficiency; CF: cystic fibrosis; GAL: galactosemia; CAH: congenital adrenal hyperplasia; BTD: biotinidase deficiency; MSUD: maple syrup urine disease; G6PDD: glucose-6-phosphate dehydrogenase deficiency.

The Mexican healthcare system is complex and fragmented, with multiple public healthcare providers serving different fractions of the population according to their employment affiliation or lack thereof. A child born in Mexico will receive medical care, including NBS, depending on the employment affiliation of their parents. Petroleos Mexicanos (PEMEX) is the Mexican national oil company and it has a network of hospitals and clinics across the country that is available to workers of the company and their families and serves about 1.2% of the Mexican population. The PEMEX Genetics Department has since 2005 implemented the most comprehensive public metabolic ENBS program in Mexico with the screening of 69 conditions initially through MS/MS, and later in 2012 with the expansion to 76 inborn errors of metabolism (IEM) disorders, SCID, and six lysosomal storage disorders (LSDs), namely Fabry, Gaucher, Pompe, Krabbe, Hurler, and Niemann-Pick diseases[6]. Between January 2005 and December 2019, PEMEX screened 65,600 newborns born within their hospital system. Of those, 806 newborns were found to have a positive screen test by ENBS and confirmed to have a genetic disorder. Of these, 779 were confirmed to have an inborn error of metabolism, 25 were positive for a lysosomal storage disorder, and 2 were found to have a congenital immunodeficiency[7]. G6PDD was the most commonly identified condition (1 in 178 newborns), followed by transient neonatal tyrosinemia (TNT, 1 in 194 newborns). A positive screening

result for any of the six lysosomal storage conditions was obtained in 1 in 1,212 newborns and CHT was identified in 1 in 2,128 newborns. The most frequent lysosomal storage disease identified by ENBS was Pompe pseudodeficiency, followed by late-onset renal Fabry disease due to a founder variant (p.Arg363His) in the *GLA* gene in the Mexican population[7]. Early identification of newborns with these conditions through the ENBS program at PEMEX has enabled improved disease management and timely therapeutic interventions through dietary substitution and supplementation, with positive outcomes observed through longitudinal follow-up at PEMEX.

Healthcare services for military members and their dependents are provided by either SEDENA (Secretaría de la Defensa Nacional) or for navy members by the SEMAR (Secretaría de Marina). Both of these institutions have implemented ENBS and screened newborns for more than 60 conditions as part of their programs. Results of SEMAR's ENBS program showed a prevalence of 1 in 651 newborns with a genetic disorder in their population, with an ENBS coverage of 99.4% of all births in their system. The most prevalent conditions detected were G6PDD, followed by CHT and CAH[8].

Since 2019, the Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE), one of the major public healthcare systems in Mexico that serves State workers and their families throughout the country, has implemented ENBS for 66 analytes and currently screens for 78 conditions. Disease prevalence and findings to date for ISSSTE's ENBS program have not been published yet. The other two major public healthcare systems, Instituto Mexicano del Seguro Social (IMSS) and Secretaría de Salud (SS), which serve the great majority of the Mexican population, screen for 7 and 6 conditions, respectively [Table 2].

More recently, advocacy efforts from the civil society, clinicians, and researchers have achieved the implementation of ENBS in Mexican states beyond specific healthcare systems. The Mexican state of Quintana Roo legislated in 2021 the implementation of ENBS for all newborns born in that state, while most recently, in 2023, the state of Guanajuato followed suit[9,10]. Although similar to the national ENBS guidelines, these reforms to the state health laws do not explicitly mention the number or list of conditions to be screened for; however, the inclusion of ENBS as part of individual state health laws is important to achieve national implementation. Additionally, since 2022, National Newborn Screening Day has been officially recognized in Mexico every June 28th to raise awareness and increase education about it in the country. Most recently and as part of these efforts to increase awareness and achieve improved implementation and coverage, effective from July 2023, results for the five types of newborn screening mandated for Mexican newborns, namely metabolic, hearing, visual, cardiac, and hip dysplasia, will be included in the national vaccination card for all infants born in Mexico [Figure 1][11]. The national vaccination card is an official government-issued document that tracks early development, wellness, and the application of compulsory immunizations for children born in Mexico from 0 to 9 years of age. Of the mandated screenings, only metabolic NBS/ENBS involves molecular confirmatory testing, while the other types of screening are merely clinical and, similar to metabolic NBS, are not implemented consistently and widely. Therefore, inclusion and mandatory report of results for these screenings are relevant to achieve homogenization in the implementation of NBS across the healthcare institutions in Mexico, and ensure that NBS is being performed for every infant born in the country regardless of parents' employment or healthcare provider affiliation.

The estimated coverage of basic NBS in Mexico was about 84% in 2018[1]; however, a major disruption and suspension of NBS occurred in 2019 due to problems with the Ministry of Health contracting in twelve Mexican states. Although only newborns born in hospitals and medical units around the country are screened, the uptake for institutional births is high in Mexico (> 90%), but this varies among states[12], with a

Table 2. Major Mexican public healthcare providers and number of conditions that are screened for in their respective NBS/ENBS programs

Healthcare provider	Births in Mexico (%)	NBS/ENBS program	Number of conditions screened
PEMEX	0.2%	ENBS (2005)	83 (76 IEMs, SCID, LSDs, Hemoglobinopathies)
SEDENA	0.5%	ENBS	> 60 conditions (IEMs, Hemoglobinopathies)
SEMAR	0.1%	ENBS	> 60 conditions (IEMs)
ISSSTE	2.0%	ENBS (2019)	78 (IEMs)
IMSS	37%	NBS	7 (CHT, CAH, PKU, BTD, GAL, CF, G6PDD)
SS	57%	NBS	6 (CHT, CAH, PKU, GAL, CF, G6PDD)

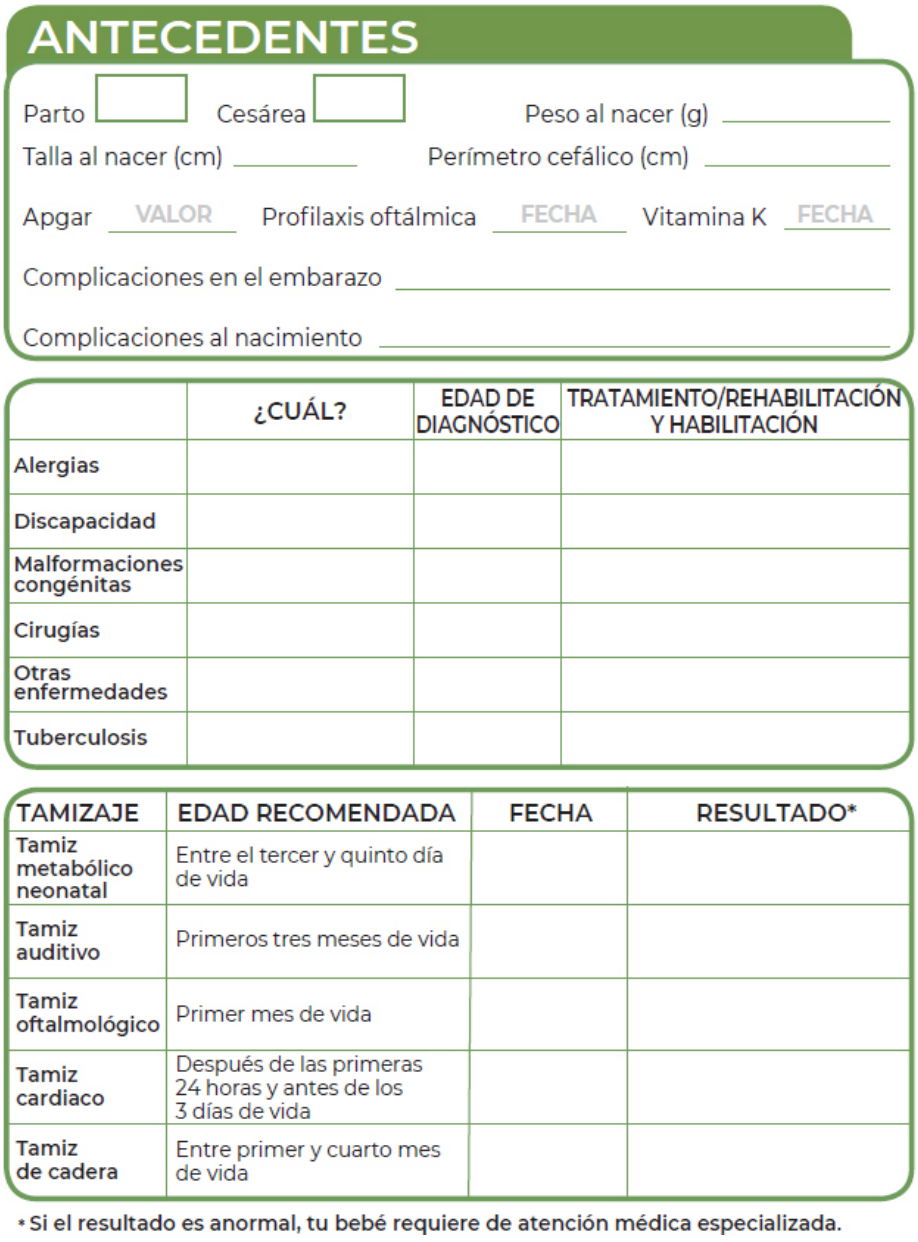
NBS: Newborn screening; ENBS: expanded newborn screening; CHT: congenital hypothyroidism; PKU: phenylketonuria; CF: cystic fibrosis; GAL: galactosemia; CAH: congenital adrenal hyperplasia; BTD: biotinidase deficiency; G6PDD: glucose-6-phosphate dehydrogenase deficiency.

fraction of newborns born at home, especially in rural areas. The approximate prevalence of 1 in 90 newborns identified with a confirmed actionable genetic inborn disorder through ENBS, highlights the need to implement expanded newborn screening programs for early disease diagnosis and management effectively across the country.

THE IMPORTANCE OF EARLY DIAGNOSIS

The importance of an early diagnosis to enable proper clinical and therapeutic management cannot be overstated. Ideally, NBS/ENBS will detect a biochemical and subsequent molecular defect in the first days of life and early enough to enable a timely intervention preventing long-term disability and mortality. A recent report from a national tertiary medical center in Mexico showed that only 35.4% of children ascertained for having an IEM detectable by ENBS had been screened at birth. Furthermore, these patients had an average diagnosis time of 4 months, which is too late to intervene for many IEMs with available interventions[13]. In contrast, ENBS programs that can detect disorders early enough and provide appropriate management and treatment are observing improved outcomes for patients with IEMs and LSDs[6,7].

Another dramatic example of the importance of early diagnosis and intervention is the recent introduction of newborn screening for Spinal Muscular Atrophy (SMA) in some countries. The emergence of novel molecular therapies for this condition, such as gene replacement via adeno-associated viral vectors (AAVs) and splicing modifying molecules, has transformed the prognosis of a condition that, if untreated, results in 90% of patients being dependent on permanent mechanical ventilation or dying within the first 2 years of life. In the last few years, three therapies have been developed and approved to treat SMA by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), while additional therapies are still in development[14]. In Mexico, the Federal Commission for Sanitary Risk Protection (Comisión Federal para la Protección de Riesgos Sanitarios, COFEPRIS) is the approving body for new drugs and therapeutics. The three available treatments for SMA are approved by COFEPRIS, but they are not necessarily available to patients in the country and their cost is prohibiting for most families. Moreover, in all cases, therapeutic success depends on the administration of the drugs at the earliest possible age and this is only possible through early diagnosis, which can be facilitated by NBS. Among SMA patients with two copies of the paralogue gene *SMN2*, 78% achieved independent walking when treated with a disease-modifying therapy within the first 30 days of life, in comparison with 25% and 8% for those treated between 31 to 90 and after 90 days, respectively. Early intervention showed an even greater impact on motor skill development in patients with three copies of *SMN2*, with 100%, 86% and 31% of children walking by themselves when treated within the same ranges described before[15].



ANTECEDENTES

Parto ☐ Cesárea ☐ Peso al nacer (g) _____

Talla al nacer (cm) _____ Perímetro cefálico (cm) _____

Apgar VALOR Profilaxis oftálmica FECHA Vitamina K FECHA

Complicaciones en el embarazo _____

Complicaciones al nacimiento _____

	¿CUÁL?	EDAD DE DIAGNÓSTICO	TRATAMIENTO/REHABILITACIÓN Y HABILITACIÓN
Alergias			
Discapacidad			
Malformaciones congénitas			
Cirugías			
Otras enfermedades			
Tuberculosis			

TAMIZAJE	EDAD RECOMENDADA	FECHA	RESULTADO*
Tamiz metabólico neonatal	Entre el tercer y quinto día de vida		
Tamiz auditivo	Primeros tres meses de vida		
Tamiz oftalmológico	Primer mes de vida		
Tamiz cardiaco	Después de las primeras 24 horas y antes de los 3 días de vida		
Tamiz de cadera	Entre primer y cuarto mes de vida		

*Si el resultado es anormal, tu bebé requiere de atención médica especializada.

Figure 1. Example of the page that includes newborn screening results in the national vaccination card in Mexico since 2023. Parents of newborns in Mexico receive a national vaccination card that is used to record all immunizations, and basic medical and wellness evaluations from 0 to 9 years of age. Since July 2023, the national vaccination card includes and must have recorded the results of the five different screenings that are performed in newborns: metabolic, hearing, visual, cardiac, and hip dysplasia. Although ENBS is now mandated in Mexico, the number and list of conditions are unspecified and variable depending on the healthcare institution of parents' affiliation.

In Mexico, SMA screening remains unavailable in the national public healthcare system (and even limited in the private sector). Consequently, disease detection relies heavily on clinical expertise, which varies significantly within the country's medical community. Despite the absence of screening for the disease and the high cost of therapies, a few patients have been able to be treated in selected reference medical centers. The first patient treated in Mexico's public system with onasemnogene abeparvovec was infused at the Children's Hospital in Mexico City (*Hospital Infantil de México Federico Gómez*) at 10 months of age.

However, this delay in diagnosis and treatment for SMA sadly resulted in the patient dying from respiratory complications a couple of months later.

THE FUTURE AND EQUITY OF NEWBORN SCREENING

The development and implementation of next-generation genomic sequencing for the study of genetic disorders and, later on, for molecular clinical diagnostics of patients with rare genetic disorders has raised the possibility of utilizing genomic sequencing as a newborn screening tool. The utilization of modern genomic sequencing technologies offers the possibility to identify actionable genetic diseases promptly before symptom onset and beyond the limitations of identifying abnormal metabolites through MS/MS in ENBS. In the last few years, several pilot programs have been implemented to evaluate the feasibility, effectiveness, and impact of genomic newborn screening (gNBS) in sick and healthy newborns primarily in the United States and the United Kingdom[16-19]. Currently, at least thirty different gNBS programs are being planned or implemented on a research basis or commercially in Europe, the US, the UK, China, and Australia[20,21]. Issues regarding how many and which conditions to include, data analysis and variant interpretation, return of results, data privacy and storage, and data revisiting and custody are among the main themes that are being explored and discussed for gNBS programs[19-21]. While promising to improve the lives of millions of newborns in the corresponding countries, the current landscape of gNBS programs further reflects the disparities in the implementation of genomic sequencing for precision health around the world. The lack of representation of non-European ancestry individuals in genomic projects and databases is now a well-recognized problem, yet slow progress has been made in expanding genomic sequencing efforts to underrepresented populations[22]. A particular issue related to this is the interpretation of genomic data and variants for individuals from underrepresented populations like those in LAC. Studies that have assessed the diagnostic efficacy of genomic sequencing in patients from different ancestries, have shown a higher proportion of variants of unknown significance and a reduction in diagnostic efficacy for patients from admixed populations such as Hispanics and African Americans in the US[23,24]. The implementation of population-level genomics programs, including gNBS, can help not only expand the characterization of human genomic variation, but also clarify the clinical significance of variants of unknown significance for individuals from underrepresented populations in both high-income (HIC) and low- and middle-income countries (LMICs).

The question is not anymore whether gNBS should be done because research programs exploring its feasibility are already being planned or implemented in several countries, but what the best practices for these programs should be, their advantages and limitations, and how to ensure the equitable implementation of genomic technologies to reduce and not broaden health disparities around the world[18,25]. While HICs are already implementing research gNBS pilot programs to assess their feasibility for public health, LMICs like Mexico and most other LAC countries are tracking behind on the implementation of genomic technologies for the research and diagnosis of genetic diseases, let alone exploring their implementation at the population level or in gNBS programs. This gap in implementation is multifactorial, but a major factor is the cost of local genomic sequencing in LMICs, which can be 3-5 times higher than in HICs due to high import fees and taxes plus distributor fees for instruments and reagents.

Mexico was the first country in LAC to initiate NBS eleven years after NBS was mandated and implemented in some states in the United States, while other LAC countries have not yet implemented basic NBS programs. Proof of concept for the need to implement ENBS in Mexico was published in 2000[26], yet more than two decades later, ENBS is not being performed for most newborns in the country. Are the countries in the LAC region going to maintain this trend and explore implementing gNBS a decade or more later than more advanced countries?

Mexico and other LAC countries should start considering now how to best implement effective and comprehensive ENBS programs for their populations and in their primarily socialized healthcare systems. At the same time, governments and health authorities across the region ought to begin considerations for the implementation of modern and future early disease detection technologies for population health. While the time for adoption and implementation of MS/MS technologies for ENBS may have passed for the countries of the region, it is now timely to explore the implementation of the next generation of ENBS programs through research pilot projects that can assess their effectiveness and feasibility. Ideally, gNBS should be complimentary to metabolic ENBS using MS/MS; however, in countries where ENBS does not exist as the standard of care for newborns nationwide, the strategic investment in technologies that can maximize the benefit for as many newborns, patients, and families as possible should be considered.

Not only will this have an impact on improved health and life expectancy outcomes for newborns at risk, but it has been proven that early detection of rare genetic diseases is cost-effective for healthcare systems and societies in the long term[27-30]. Additionally, the ability to know the prevalence of diseases in local populations and the number of patients with conditions for which there is an available treatment can enable better national public health planning, budgeting, and price negotiation with pharmaceutical companies for improved access to treatments and proper disease management. In healthcare systems marked by limited resources and challenging access to new therapeutics for rare diseases, early diagnoses and interventions become imperative. Patients treated at advanced stages of a disease are at greater risk of long-term complications and disability, which not only profoundly impacts the patients and their families, but also the already constrained healthcare budgets and systems in resource-limited countries such as Mexico and those in the LAC region.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and writing of the original manuscript: Gonzaga-Jauregui C, Moreno-Salgado R, Tovar-Casas J, Navarrete-Martínez JI

Provided insightful comments and revised the manuscript: Gonzaga-Jauregui C, Tovar-Casas J, Navarrete-Martínez JI

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Opinion

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Incorporating a new disease in the newborn screening programs in Europe: the spinal muscular atrophy case study

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Abstract

Patient advocacy organizations have a forefront role in ensuring that patients' voices and needs are embedded as a constitutive basis in drug development, diagnosis, and policy recommendations in the healthcare ecosystem. Their sustained involvement in accelerating the policy changes for inclusion of additional diseases in the newborn screening (NBS) programs, supporting harmonization in terms of number of screened diseases across the European Union, constitutes a driving force for advancing the quality of care and the management of rare diseases by aligning NBS policies and practices internationally. In the current European landscape, NBS varies significantly across regions and countries. Patient advocacy organizations are acting to alert healthcare authorities of the existing inequity in NBS and recommending that additional diseases be added to the national NBS programs. Here, we describe the state of play for Spinal Muscular Atrophy (SMA) as a model for advancing NBS for rare diseases where a treatment regime is available. Ultimately, a broad understanding of NBS for SMA will additionally serve as a means to understand the financial impact of early therapeutic intervention for a rare disease.

Keywords: Newborn screening, rare diseases, patient advocacy organizations, spinal muscular atrophy

INTRODUCTION

Across countries, newborn screening (NBS) is implemented heterogeneously, with significant variations between high- and low-middle-income countries. A study published by Novartis and Charles River Associates (CRA) in March 2022 showed that one heel prick test can potentially diagnose up to 50 diseases, as demonstrated by Italy, which ranks as the first country in the European Union (EU) where NBS is currently implemented for 48 diseases (or more, depending on the region), while Cyprus and Romania perform NBS for only two diseases[1]. The efficiency of the diagnostic pathway remains a critical step in the management of SMA, and the inclusion of SMA in the national NBS programs, with adequate follow-up and genetic counseling, facilitates earlier treatment access and care. Across Europe, several countries have already included SMA in their national screening programs, among them Belgium, Germany, Netherlands, and Poland, while others developed pilots and provided an efficient reimbursement system for access to diagnostic resources (Austria, Czech Republic, Denmark, Finland, France, Italy, North Macedonia, Spain, Sweden, and the United Kingdom)[2,3].

Spinal Muscular Atrophy (SMA) is a genetic recessive disease caused by the mutation or absence of the Survival Motor Neuron 1 (SMN1) gene located in the q region of chromosome 5, often referred to as 5q SMA, which results in motor neuron degeneration and thus progressive muscle paralysis. In recent years, new treatments have been shown to significantly improve the motor function of symptomatic patients, but do not enable them to regain normal motor activity. In adults, they stabilize or even slightly reverse the course of the disease. Their effectiveness is largely dependent on the age of the patient and his or her ability to renew motor neurons, but above all, on the level of degeneration of the nervous system[4]. Three effective treatments have received regulatory approval from the European Medicines Agency (EMA) and at least one of them is now available for infants in some European countries[5]. Disease severity is a continuum; however, to facilitate the standard of care, the disease forms were classified by type (type 0 to 4) based on the age of disease onset[6]. The age at which a child first exhibits symptoms is a reliable predictor of the progression of the disease in terms of motor functions and associated disabilities if left untreated [Table 1].

For patients diagnosed through a genetic test of 5q13 SMA, a double deletion of the SMN1 gene will indicate a positive result. SMN1 and SMN2 have identical sequences except for a single nucleotide change in exon 7 of SMN2 which alters splicing. SMN2 usually produces a small amount of functional protein and the copy number of SMN2 typically influences the severity of SMA disease[4].

• 1 copy of SMN2: the child will develop a severe form of type 1 and will probably show clinical signs from birth;

• 2 copies of SMN2: the child will present the first symptoms at a very young age and will probably have SMA type 1 or 2;

• 3 copies of SMN2: first symptoms develop in childhood, and the patient will probably have SMA type 2 or 3;

• 4 copies of SMN2: the first symptoms will appear in adolescence or adulthood, and the patient will probably develop a type 3 or 4 SMA;

• Beyond 5 copies is quite rare, the patient can develop SMA type 4, and many adults diagnosed in this way are usually asymptomatic[5].

Table 1. Characteristics of 5q SMA types

	Type 1	Type 2	Type 3	Type 4
Age of onset of first symptom	Before 6 months	Between 6-18 months	After 18 months	Adult age (> 18 years)
Mobility status without treatment	Non-Sitter	Sitter	Walker	Walker
% of Incidence	50%*	25%	22%	3%

*Incidence of type 1 is quite high, but because of the short life expectancy (less than 2 years, median life expectancy 12 months) of this population, prevalence of type 1 remains quite low.

The published clinical studies results have shown that the effectiveness of all approved treatments is significantly higher when the child is treated before the onset of the first clinical sign[5-7]. Three treatments are approved in Europe, targeting the survival motor neuron gene (SMN1 and SMN2) production:

(1) Spinraza™ (nusinersen), an antisense oligonucleotide (ASO) targeting the SMN2 gene, administered via intrathecal injection every 4 months[8];

(2) Evrysdi™ (risdiplam), SMN2 splicing modifier overexpressing SMN production, medicine administered per os (PO), once per day[9];

(3) Zolgensma™ (onasemnogene abeparvovec), gene therapy, adding the SMN1 sequence, single-dose intravenous infusion[10].

Why include SMA in NBS programs?

During the clinical studies preceding the marketing submission or authorization of new treatments, in addition to symptomatic patients, pharmaceutical companies conducted a series of trials on patients diagnosed with SMA at birth via genetic screening. Most investigations were focused on patients treated presymptomatically who had two or three copies of SMN2 and who were predisposed to developing an early form of the disease. In the NURTURE study, a clinical trial conducted by Biogen, the newborns were administered intrathecal nusinersen (Spinraza™) during neonatal development, with long-term follow-up to evaluate the benefits on survival, respiratory interventions, and motor outcomes and if the treatment guaranteed a favorable safety profile. Interim results from the NURTURE study concluded that, after a follow-up of 2 years and 9 months, there were clear clinical benefits for the infants with two or three copies of the SMN2 gene who received treatment, providing an opportunity for early intervention and improvement of symptoms experienced. The ENDEAR study evaluated the motor-milestone responses and the event-free survival in infants with SMA treated with nusinersen compared with a control group. An interim analysis showed a better motor-milestone response in the group treated with nusinersen, which led to the early termination of the trial. The final analysis showed a significant improvement in motor response and survival in the arm treated with nusinersen compared with the control group[5,8].

A comparable level of efficacy has been demonstrated by Zolgensma (onasemnogene abeparvovec), the gene therapy medication developed by Novartis Gene Therapies, that was administered in a single-arm SMA patients trial who had two or three copies of SMN2. The results highlighted that 7 out of 14 (50%) infants with two SMN2 copies had gross motor performance similar to normal development, while all 14 (100%) infants had fine motor performance similar to normal development[10]. Moreover, all patients with three copies of SMN2 treated presymptomatically achieved the primary endpoint, demonstrating the ability to stand without support during the 2-year follow-up visit. More recently, Evrysdi (Roche) also showed a comparable level of efficacy based on the results presented in their publication of the RAINBOWFISH trial

result, showcasing that 80% of the infants involved in the trial were able to sit without support for at least 5 s after one year of treatment[11-13].

In February 2020, at the SMA Europe Scientific Congress (Évry, France), SMA patient organizations and leading clinicians in the field expressed their concern about an important loss of opportunity for early intervention for infants not screened at birth. Subsequently, they created the European Alliance for SMA NBS. The goal of this Alliance is to accelerate the inclusion of SMA in the NBS program in Europe. It emphasizes that delays in adding SMA to the screening programs could result in children not being identified early enough, thus missing out on available life-saving treatments.

The Alliance has developed tools to assist clinicians and national patient organizations in advocating for the implementation of NBS screening in their countries, such as flyers or posters to support the advocacy actions, and developed a white paper that addresses most of the questions related to access to diagnosis, treatments, and care, and that, at the same time, may encourage the introduction of SMA screening in the national programs in Europe.

How does SMA address the Wilson & Jungner criteria and why should it be included in all NBS programs

The Wilson and Jungner criteria were first published in 1968, and it comprises 10 principles of population screening with the scope of guiding the screening decisions[14-16]. The principles are explained below in Table 2, together with arguments to screen for SMA at birth based on each criterion.

In reality, the inclusion of SMA screening in NBS programs in Europe has been moderately successful so far. In 2020, less than 25% of the babies born in the EU were screened, and this rate drops to 15% in continental Europe[22]. In comparison, three years after SMA was added to the federal recommended list of diseases for screening at birth, 98% of the newborns in the United States of America are now screened for SMA at birth[23].

Main obstacles encountered - cumbersome, redundant and complicated administrative procedures

The implementation of NBS in the EU member states is disparate, and sometimes, as it happens in Spain or Italy, it is a decision at the regional level. This multiplies the number of dossiers to be submitted, sometimes with contradictory analyses from one country to another. In order to facilitate the constitution of the different dossiers, the Alliance has produced a white paper answering the main questions asked by the national or regional agencies with the associated scientific references[22].

The national procedures for implementing screening might sometimes be unclear, not fully transparent and therefore difficult to understand for many stakeholders including patient associations. The Alliance, with the support of the CRA, has carried out a mapping of the different procedures by country and identified points of contact, as well as a series of good practices or success stories based on the feedback received. This study shows that in some countries, there is no formal procedure and usually, in a vast majority of them, a patient association is not authorized to initiate a request for NBS for a disease. The patient advocacy associations are sometimes consulted during the development of a new series of procedures, but in most cases, their input remains minimal, and it is not captured either through formal or informal meetings[22].

Data privacy concerns

Genetic data are considered sensitive data according to the EU General Data Protection Regulation (GDPR). Furthermore, many countries, including France, have a very strict legislation regarding the protection of genetic data of their citizens. Consequently, the implementation of genetic screening requires a specific national legislative change.

Table 2. Why SMA should be screened at birth based on Wilson & Jungner criteria

Wilson & Jungner criteria	Argument for SMA
1. The condition sought should be an important health problem	Half of the children affected by the disease die before the age of 2 years old
2. There should be an accepted treatment	Three treatments are showing efficiency And all trials have demonstrated improved efficacy if administered presymptomatically
3. Facilities for diagnosis and treatment should be available	Monogenic diseases due to double deletion of the SMN 1 gene
4. There should be a recognized latent or early symptomatic stage	Floppy Infant Syndrome
5. There should be a suitable test or examination	Genetic Polymerase Chain Reaction (PCR) test is available[17]
6. The test should be acceptable to the population	Early treatment prevents the death of babies or permanent disabilities
7. The natural history of the condition should be adequately understood	The natural history of the disease is published and well documented[18,19]
8. There should be an agreed policy on whom to treat as patients	Scientific publications recommend whom to treat[9,20]
9. The cost of case-finding should be economically balanced	Cost-effectiveness studies show efficiency in all countries where they were performed[21]
10. Case-finding should be a continuing process	SMA is a recessive disease, and NBS should be applied permanently to identify new cases

In many European countries, dried blood samples are stored after the NBS test is performed for research purposes. However, the parents or caregivers need to express their consent (opt-in or opt-out) for the sample to be stored or destroyed, de-identified, used for further scientific purposes, or shared through different research platforms. Secondary use of data would be possible in collaboration with the European biobank infrastructures which can oversee the legal requirements and privacy protection regulations and compliance with GDPR and national legislation. Moreover, as data sharing and re-use may provoke certain concerns related to breach of privacy and stigmatization, it is essential to name the safeguards that are in place pre- and post-procedures. An alignment in executing the NBS programs across Europe would be possible if it is offered as a service respecting the legal provisions and funded publicly, following a specific consent related not only to the immediate benefit for the infant, but also to the research results and possible applications, such as in the identification of novel biomarkers for SMA. Specifically, analyzing the link to disease progression or predicting individual responses to therapy would enable further clarifications on the disease pathogenesis and therapeutic response.

Country-specific pilot initiatives for including a new disease in the national NBS programs

Recently, many countries have expressed their wish to develop a pilot program, often targeting a subset group of their population, as a prerequisite to the national implementation of including a new disease within their NBS program. This practice is certainly useful for the countries introducing a new disease into their screening panel, but for SMA, the pilots conducted in Germany[24] or Belgium[25], as documented in scientific publications, have displayed the inequalities between the citizens of these countries. In Belgium, for example, the Wallonia region implemented screening very quickly at the beginning of 2020, whereas the Flemish part initiated it in 2022. The existence of a country-specific coalition, along with the sustained exchange of knowledge, perspectives, procedures, and strategies, is essential to diminish the possible variations encountered per region of the same country.

Implementation of genetic NBS

In several countries, SMA screening is the first to be carried out through genetic analysis[6,26], and it has raised numerous questions from the local health authorities on both technical approaches and ethical issues.

Patient organizations consulted the learnings and facts presented by the International Society for Neonatal Screening (ISNS), and in 2020, a low level of interest was noted for screening via biological analyses. Consequently, the Alliance has tried to address this issue by organizing an online webinar to get technical answers from the genetic testing manufacturers and, at the same time, to clarify the effectiveness and the risks of false negatives and false positives, and to remove any ambiguity about the eugenic nature of this type of testing[27].

Screening findings

NBS is a fundamental public health service that identifies a potential risk for developing a rare or very rare disease. Within the framework of SMA, as each country produces or tries to produce its own genetic analyses, it becomes almost impossible to have a harmonized analysis across European countries. The number of cases remains insufficient to support a comparative analysis, as the studies developed in different countries often have conflicting results and are based on scattered data. The Rare Barometer developed by EURORDIS-Rare Diseases Europe, under the collaborative Innovative Medicines Initiative (IMI 2 JU) Screen4Care, is an open survey available in 23 languages, which can be accessed by anyone in the world suffering from a rare disease, carriers, or family members of patients. This survey systematically collects the patients' opinions on transversal topics, aiming to translate them into key facts and figures that can be shared with a wider public, including policy and decision makers, thereby ensuring direct patient involvement in exemplifying the topics that matter most to them[28].

The Rare 2030 Survey, initiated by EURORDIS-Rare Diseases Europe to explore the future of rare disease policy and conducted through the Rare Barometer program, received 3,998 responses from all over the world, and highlighted the growing interest among patient organizations to be directly involved in the research process. The report underlines that only 18% of the responders (representing patients living with a rare disease) had been previously involved in the development of treatments and therapies, and one of the reasons causing this is the lack of public/private funding for small populations. Two hundred fifty-two patient representatives expressed their willingness to contribute directly throughout the research process, including helping researchers recruit participants for the clinical trials, reviewing research proposals to ensure an alignment with patients' needs, actively participating as partners or co-investigators, contributing to raising funds, and actively disseminating information about the project research and its results, all of which would bring clear benefits including for SMA patients and their families. Being actively involved in the research process as an equal partner or co-investigator would ensure better dissemination of the available resources, knowledge sharing, and cross-border alignment in multidisciplinary care for SMA patients[29].

Strategies for disease prevention, detection, and treatment represent one of the priorities of the International Consortium on Newborn Sequencing (ICoNS), a global alliance network founded in 2022 by leaders from eight sequencing projects (BabySeq, Genomics England, GUARDIAN Study, BeginNGS, Early Check, Screen4Care, ScreenPlus, and BabyBeyond) that tries through its annual conferences to represent the vision of various international stakeholders on the implementation of NBS as a public health measure[30]. The Alliance brought to attention that a robust application of the NBS programs can be achieved only through a common understanding and coordination among the parties involved. This can be sustained through the development of an effective infrastructure adapted to the population's necessities and harmonized with the industry precompetitive challenges. An ICoNS working group has been tasked with creating a functional mechanism for ensuring documentation consistency, alignment of terminology and metrics, with the final objective of consolidating data results and facilitating the data sharing in the consortium[30,31].

Cost-effectiveness analysis

Because of the high cost of treatment of Evrysdi™, Spinraza™, or Zolgensma™, the issue of the cost of genetic screening and the additional cost of treatment is often not prioritized by the health authorities[4,32]. Several studies analyzed the cost-effectiveness of implementing NBS, based on different case studies. In a study carried out in England, the introduction of SMA screening identified approximately 56 infants suffering from SMA yearly, accounting for 96% of cases, reporting savings of £62,191,531 and quality-adjusted life years of 529, compared with a scenario where NBS would not have been implemented[33]. In the Netherlands, another study highlighted the cost-utility model that estimates the lifetime health impacts and costs for identifying SMA, compared with a pathway without NBS, concluding that in the cohort studied (17 patients), the number of quality-adjusted life-years was approximately 320 years, while the total healthcare cost decreased by €12,014,949[34]. In Belgium, the lifetime cost-effectiveness showed a minimal increased economic cost for healthcare services (€ 6,858,061 *vs.* € 6,738,120), but more quality-adjusted life years, compared with a scenario of "no screening" (40.95 *vs.* 20.34), concluding that screening of SMA, accompanied by early-stage treatment, is more cost-effective and it represents a comprehensive choice from a societal perspective[35].

Newborns with fewer than four SMN2 copies typically receive partial reimbursement for treatment costs from national authorities. In contrast, babies identified with double deletions of SMN1 and 4 or more copies of SMN2, who also face a heightened risk of developing a late-onset form of SMA, have difficulties in obtaining treatment coverage.

Is the solution developed by SMA Europe by creating the alliance to support national organizations reproducible for other rare diseases?

With the development and approval of new therapeutic agents for an increasing number of rare diseases, it is increasingly important to add new diseases to national NBS programs. A number of therapies now exist for Duchenne Muscular Dystrophy (DMD) including gene therapy (Elevidys™), antisense drugs (Ataluren™, Eteplirsen™, Viltolarsen™, Casimersen™), glucocorticoids (Deflazacort™, Vamorolone™), and Histone deacetylase inhibitors (Givinostat™). While the typical age of onset is around 4 years of age, many patients may benefit from early screening to ensure that therapies can be administered to reduce muscle loss[36]. Other examples where early intervention would benefit patients are those with inherited retinal diseases such as Leber congenital amaurosis or retinitis pigmentosa for which Luxturna™ (Voretigene neparvovec) has been approved, but only in patients with enough functioning cells in the retina[37].

The work conducted by the Alliance has demonstrated that an alliance is necessary to accelerate the implementation of NBS for SMA and could be replicated for other diseases, which might contribute to accelerating the expansion of NBS programs in Europe.

The World Health Organization guideline on the "Standards for improving the quality of care for small and sick newborns in health facilities" acknowledges that "every child has the inherent right to life" (Art. 6) and mentions that the goal is to "strive to ensure that no child is deprived of his or her right of access to such health care services" and they should pursue full implementation of this right to diminish infant and child mortality and to develop a preventive healthcare system (Art. 24)[38,39]. The guidance highlights the need to respect, protect, and fulfill the newborns' rights without any discrimination. However, despite sustained international efforts to align different roles and responsibilities and to ensure a transparent and robust model, the processing of requests at local per country or per region levels remains constrained by different factors (absence of registries, qualified personnel, availability and complexity of diagnostic services, healthcare expenditure, lack of clear procedures, *etc.*).

Is it possible to have a European strategic recommendation for inclusion?

In the USA, the Advisory Committee on Heritable Disorders in Newborns and Children recommended a Uniform Screening Panel to ensure the adequacy and uniformity of the evaluation programs, meant to overcome various barriers and establish a national framework for scientific evaluation of conditions, standardization of cases and reporting, better oversight of implementation procedures, data collection, surveillance and re-use, as well as addressing the financial needs to deliver the relevant services to a wider population[40]. In Europe, patient advocacy organizations, with technical and scientific support from the EMA, could propose recommendations for the inclusion of a new pathology in the panel of EU states, but in this case, each state should define clear rules for meeting certain criteria to achieve the expected outcomes.

When faced with a lack of procedure, those wishing to propose the inclusion of a new condition are often confronted with a missing action plan in terms of: who are the stakeholders that can initiate an application? what scientific data and evidence should be provided to endorse it? what is the validation process for inclusion of a new disease in the national panel? and what is the usual timeframe for handling such requests?

An international alignment at the European level for SMA could improve equitable access in the provision of NBS and ensure that newborns receive a qualitative screening for multiple diseases regardless of their nationality, race, or socioeconomic background. Creating an ecosystem that gathers all experiences reported, initiatives, and diagnostic approaches would move forward in the alignment of NBS screening programs, and would diminish the current discrepancies and limitations that exist between countries. However, taking into account the complexity of implementation in real-world settings, with the development of accessible pathways, changes related to ethical and informed consent, data and scientific sharing, including linkage of registries, the process of introducing new conditions in the national screening panels remains difficult at this stage.

Is it necessary to have an innovative interconnected database?

To have more reliable statistical analysis, it will be necessary to gather all results with a pathophysiological description in a single database. The European Reference Networks (ERNs) could play an important role in centralizing the data at the European level. An identical analysis at the level of the other continents would be desirable. Ideally, the interconnection of these data could advance the understanding of the disease and serve as a basis for economic benefit-risk analyses.

CONCLUSION

There are still many areas for improvement to speed up and facilitate the procedures for including a new condition in NBS programs in the European member states. A stronger commitment from countries and healthcare organizations is essential for addressing the bottlenecks experienced by patients and their caregivers. The EU can set the example by endorsing genetic screening as a fair procedure in terms of human rights, and can take part in deciding if the screening techniques can be approved for more than one disease, while EMA can play a role by providing treatment recommendations that have been proved to be successful in the past and showed efficacy when administered presymptomatically. The engagement of different stakeholders, especially the direct involvement of patients and patient advocacy organizations across all NBS-related activities, remains a critical point in public health discussions, not limited just to ethical or societal concerns, but also in connection with the advancement and harmonization of equitable access to screening techniques and treatment options. Learnings from SMA therapy development and the introduction of NBS in SMA in a consistent and European-wide manner can serve as a model for additional rare diseases in the future.

DECLARATIONS

Authors' contributions

Manuscript concept: Ouillade MC, Tătaru EA
Contributed to the first and subsequent drafts: Tătaru EA, Ouillade MC
Contributed to multiple critical revisions: Tătaru EA, Chan CH, Pearce DA

Availability of data and materials

Not applicable.

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Conflicts of interest

Pearce DA is an Editorial Board member of *Rare Disease and Orphan Drugs Journal*, while the other authors have declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Before you decide to publish with *Rare Disease and Orphan Drugs Journal* (*RDODJ*), please read the following items carefully and make sure that you are well aware of Editorial Policies and the following requirements.

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The topic of the manuscript must fit the scope of the journal. Please refer to Aims and Scope for more information.

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All submissions are required to be presented clearly and cohesively in good English. Authors whose first language is not English are advised to have their manuscripts checked or edited by a native English speaker before submission to ensure the high quality of expression. A well-organized manuscript in good English would make the peer review even the whole editorial handling more smoothly and efficiently.

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If an accepted manuscript was funded by National Institutes of Health (NIH), the authors may inform Editors of the NIH funding number. The Editors are able to deposit the paper to the NIH Manuscript Submission System on behalf of the authors.

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A cover letter is required to be submitted accompanying each manuscript. It should be concise and explain why the study is significant, why it fits the scope of the journal, and why it would be attractive to readers, etc.

Here is a guideline of a cover letter for authors' consideration:

In the first paragraph: include the title and type (e.g., Original Article, Review Article, Case Report, *etc.*) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;

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There is no restriction on the length of manuscripts, number of figures, tables and references, provided that the manuscript is concise and comprehensive. The journal publishes Original Article, Review, Meta-Analysis, Case Report, Commentary, *etc.* For more details about paper type, please refer to the following table.

Manuscript Type	Definition	Word Limit	Abstract	Keywords	Main Text Structure

Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	5000 max	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Review	A review article should provide readers with an in-depth understanding of a field by summarizing existing literature, and highlight key gaps and challenges to address future research.	7000 max	Unstructured abstract. No more than 250 words.	3-8 keywords	The main text may consist of several sections with unfixed section titles. We suggest that the author includes an "Introduction" section at the beginning, several sections with unfixed titles in the middle part, and a "Conclusion" section in the end.
Meta-Analysis	A Meta-Analysis is a statistical analysis combining the results of multiple scientific studies. It is often an overview of clinical trials.	5000 max	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Systematic Review	A Systematic Review collects and critically analyzes multiple research studies, using methods selected before one or more research questions are formulated, and then finding and analyzing related studies and answering those questions in a structured methodology.	3000 max	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
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Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	2500 max	Unstructured abstract. No more than 250 words.	3-8 keywords	/
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2.3 Manuscript Structure

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The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

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Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email

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Highlights are mandatory because they can help increase the discoverability of your article through search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). They should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters per bullet point, including spaces).

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Original research, systematic reviews, and meta-analyses require structured abstracts. The abstract should provide the context or background for the study and should state the study's purpose, basic procedures (selection of study participants, settings, measurements, analytical methods), main findings (giving specific effect sizes and their statistical and clinical significance, if possible), and principal conclusions. It should emphasize new and important aspects of the study or observations, note important limitations, and not overinterpret findings. Clinical trial abstracts should include items that the CONSORT group has identified as essential. It is not allowed to contain results which are not presented and substantiated in the manuscript, or exaggerate the main conclusions. Citations should not be included in the abstract.

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The graphical summary is optional. It should summarize the content of the article in a concise graphical form. It is recommended to use it because this can make online articles get more attention. The graphic abstract should be submitted as a separate document in the online submission system. Please provide image with a resolution greater than 300 dpi. Preferred file types: TIFF, PSD, AI, JPEG and EPS files.

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The introduction should contain background that puts the manuscript into context, allow readers to understand why the study is important, include a brief review of key literature, and conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved. Relevant controversies or disagreements in the field should be introduced as well.

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Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

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This section should contain the findings of the study and discuss the implications of the findings in context of existing research and highlight limitations of the study. Future research directions may also be mentioned. Results of statistical analysis should also be included either as text or as tables or figures if appropriate. Authors should emphasize and summarize only the most important observations. Data on all primary and secondary outcomes identified in the section Methods should also be provided. Extra or supplementary materials and technical details can be placed in supplementary documents.

2.3.2.4 Conclusions

It should state clearly the main conclusions and include the explanation of their relevance or importance to the field.

2.3.3 Back Matter

2.3.3.1 Acknowledgments

Anyone who contributed towards the article but does not meet the criteria for authorship, including those who provided professional writing services or materials, should be acknowledged. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgments section. This section is not added if the author does not have anyone to

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Each author is expected to have made substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data, or the creation of new software used in the work or have drafted the work or substantively revised it.

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2.3.3.6 Ethical Approval and Consent to Participate

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Studies involving animals and cell lines must include a statement on ethical approval. More information is available at Editorial Policies.

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2.3.3.7 Consent for Publication

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References should be numbered in order of appearance at the end of manuscripts. In the text, reference numbers should be placed in square brackets and the corresponding references are cited thereafter. If the number of authors is less than or equal to six, we require to list all authors' names. If the number of authors is more than six, only the first three authors' names are required to be listed in the references, other authors' names should be omitted and replaced with "et al.". Abbreviations of the journals should be provided on the basis of Index Medicus. Information from manuscripts accepted but not published should be cited in the text as "Unpublished material" with written permission from the source.

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Types	Examples
Journal articles by individual authors	Weaver DL, Ashikaga T, Krag DN, et al. Effect of occult metastases on survival in node-negative breast cancer. *N Engl J Med* 2011;364:412-21. [PMID: 21247310 DOI: 10.1056/NEJMoa1008108]
Organization as author	Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002;40:679-86. [PMID: 12411462]
Both personal authors and organization as author	Vallancien G, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1,274 European men suffering from lower urinary tract symptoms. *J Urol* 2003;169:2257-61. [PMID: 12771764 DOI: 10.1097/01.ju.0000067940.76090.73]
Journal articles not in English	Zhang X, Xiong H, Ji TY, Zhang YH, Wang Y. Case report of anti-N-methyl-D-aspartate receptor encephalitis in child. *J Appl Clin Pediatr* 2012;27:1903-7. (in Chinese)
Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. *BJOG 2018*; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
Books	Sherlock S, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm. [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
Unpublished material	Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci U S A*. Forthcoming 2002.

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Authors must keep data anonymized. If participants' details are not to be anonymized, authors must ensure that written informed consent, including consent for publication, was obtained from each participant, and consent statement must be included in the manuscript.

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9.1 Initial check

9.1.1 Initial manuscript check

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