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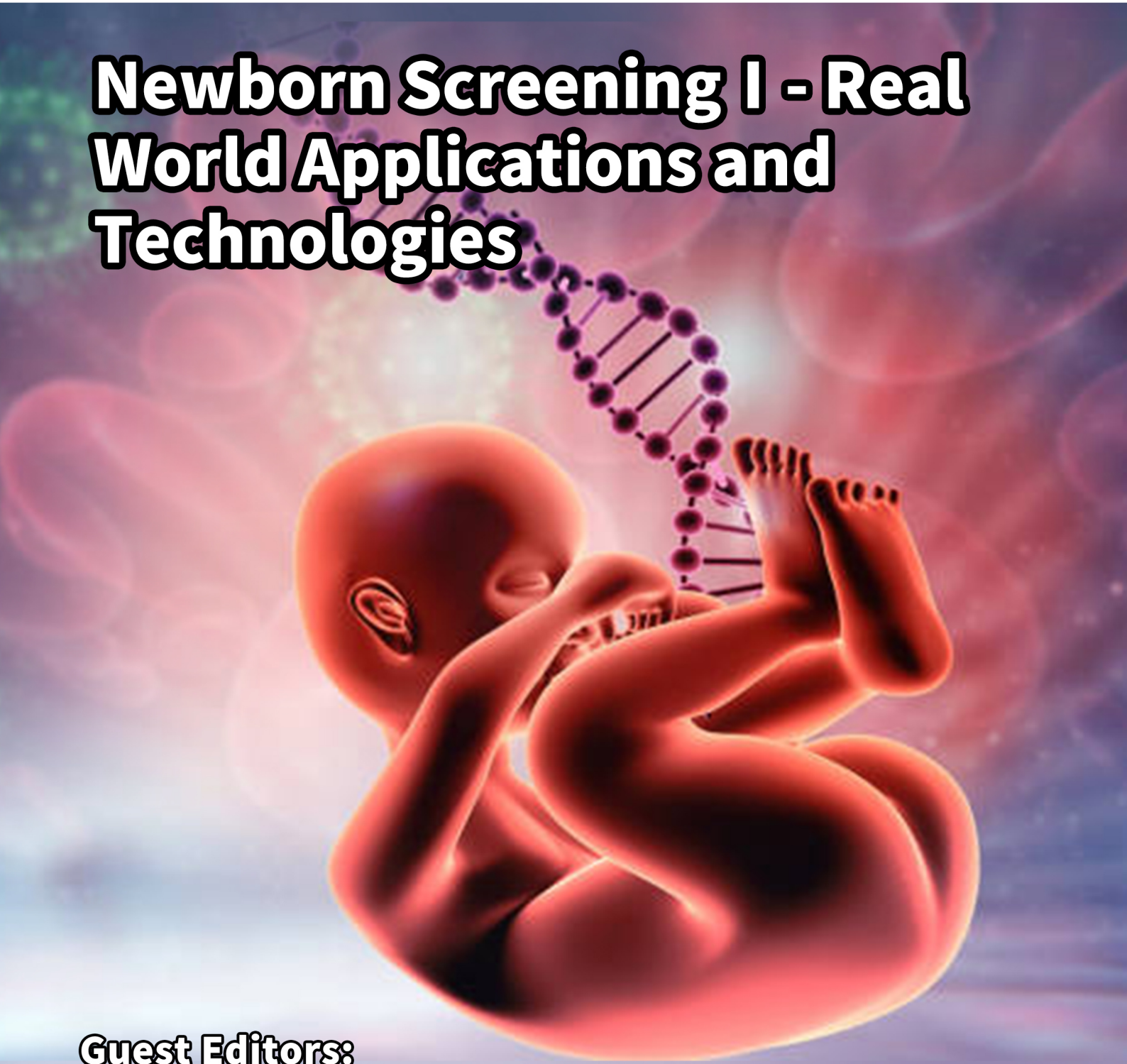
RDODJ Rare Disease and Orphan Drugs Journal

Newborn Screening I - Real World Applications and Technologies



Guest Editors:
David A Pearce, MD, PhD (Sanford Health)
Chair of International Rare Diseases Research Consortium (IRDiRC)
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
Department of Pediatrics, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD, United States.

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.

Dr. Virginie Bros-Facer
Illumina, Evry, France.

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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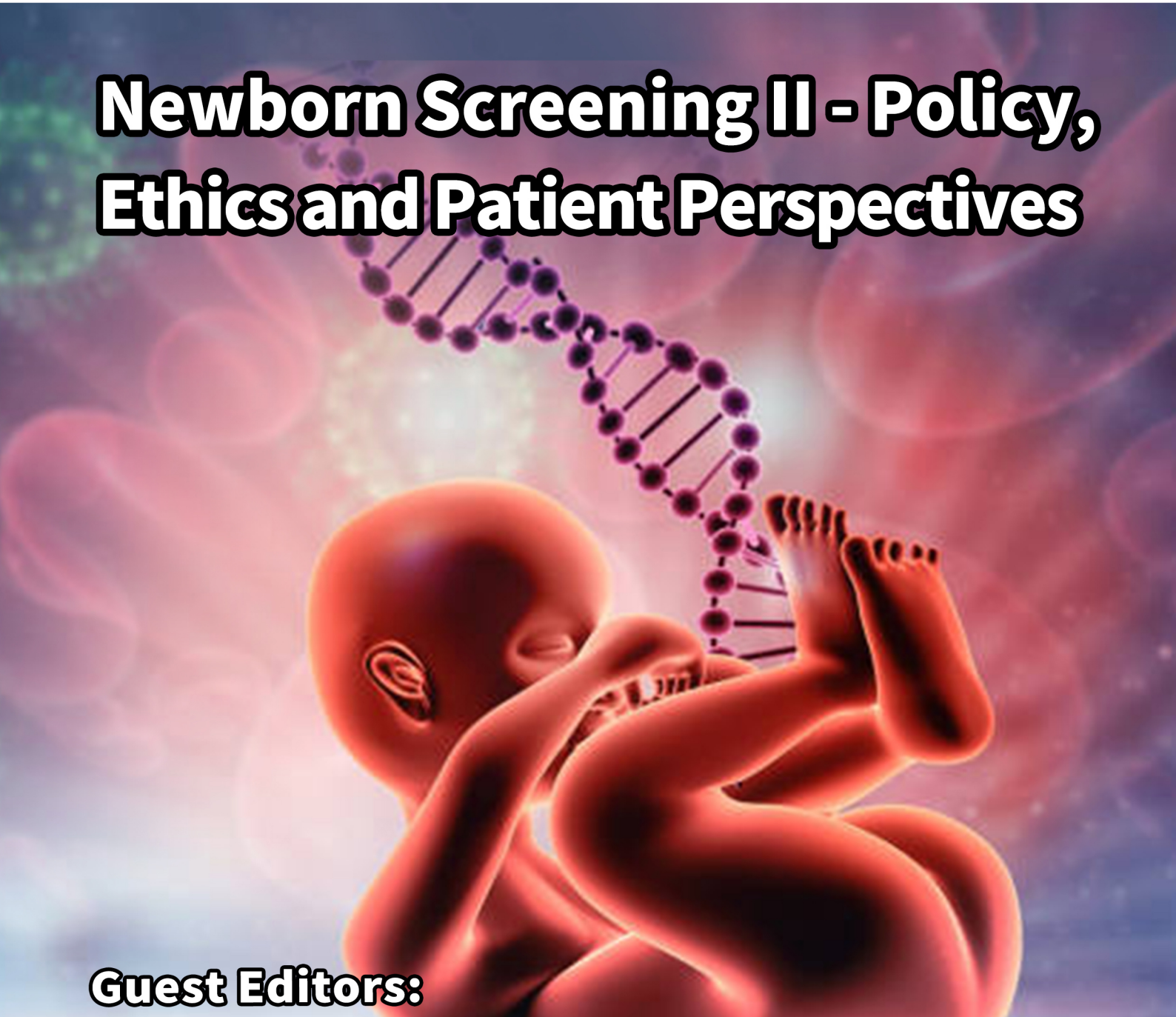
Newborn Screening II - Policy, Ethics and Patient Perspectives



Guest Editors:

David A Pearce, MD, PhD (Sanford Health)
Chair of International Rare Disease Research Consortium (IRDiRC)

Helen Malherbe, PhD (Rare Disease South Africa)
Director of Research & Epidemiology

Mary Wang, PhD (Rare Diseases International(RDI))
Programme Director of RDI

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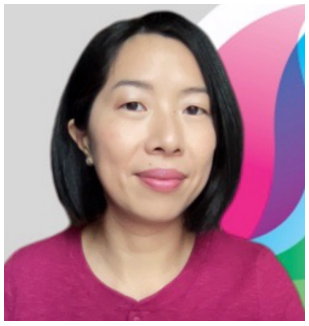
OAE

Topic: Newborn Screening II - Policy, Ethics and Patient Perspectives

Dr. Helen Malherbe

Rare Diseases South Africa, Johannesburg, South Africa.

Dr. Malherbe is a director of Rare Diseases South Africa (RDSA) and heads up the research and epidemiology portfolio there. She completed 4 years of postdoctoral research at the University of KwaZulu-Natal, Durban, South Africa, following on from her 2017 Ph.D. on the renewed need for the care and prevention of congenital disorders. She won RDSA's Rare Diamond in Advocacy award in 2018 and continues to focus on building an evidence base to inform advocacy for improved genetic services in the country. To date, her research has focused on quantifying the burden of disease represented by rare diseases and congenital disorders, and this will extend to an economic evaluation of genetic services at a new academic institution later in 2021. She supervises both Ph.D. and Master's students and continues to expand her portfolio in the peer-reviewed literature. Dr. Malherbe is an active member of relevant international and local bodies, including key World Health Organization panels and committees and the South African Department of Health Human Genetics Technical Working Group. She became part of the rare disease community in 2004 after the loss of her first child to Trisomy 18 (Edwards syndrome), establishing a contact point for families affected by the same condition.



Dr. Mary Wang

Rare Diseases International, Paris, France.

Dr. Mary Wang is Programme Director at Rare Diseases International (RDI) where she is leading initiatives on access to diagnosis and essential therapies, and oversees RDI's collaboration with the World Health Organization. Mary has 17 years of experience in biomedical research, funding, infrastructure, and policy in rare diseases. Prior to joining RDI, Mary worked at Fondazione Telethon Italy and managed international activities, advocated for research in global consortia including the International Rare Diseases Research Consortium (IRDiRC) and in the executive committee of International Consortium for Personalised Medicine (ICPerMed). Mary worked on European Commission funded projects (EJP RD, RD-Connect), leading on creation of rare disease biobank network, flagship tools for data-sharing, training for scientists and patients. At Telethon she also supported establishment of multi-million funding programmes, managed peer-review of research grants on inherited immunological and blood disorders. Mary received her PhD from University College London, UK.

Special Issue Introduction

The International Rare Diseases Research Consortium (IRDiRC) was launched in 2011 by a joint effort between the European Commission and the US National Institutes of Health with the vision to enable, through research, all people living with a rare disease to receive an accurate diagnosis, care, and available therapy within one year of coming to medical attention.

To accomplish this ambitious task, IRDiRC has established 3 main goals:

Goal 1: All patients coming to medical attention with a suspected rare disease will be diagnosed within one year if their disorder is known in the medical literature; all currently undiagnosable individuals will enter a globally coordinated diagnostic and research pipeline.

Goal 2: 1000 new therapies for rare diseases will be approved, the majority of which will focus on diseases without approved options.

Goal 3: Methodologies will be developed to assess the impact of diagnoses and therapies on rare disease patients.

Though much progress has been made towards meeting these goals, much work remains ahead. It is becoming increasingly apparent that newborn screening (NBS) is our greatest weapon in identifying rare diseases early but major challenges exist in implementing national screening programs for all newborns. New emerging technologies, such as next-generation sequencing (NGS), offer the opportunity to screen for genetic disorders through a single test but can pose a challenge in cost, complexity and access to appropriate equipment. In addition, there are ethical considerations of screening newborns for diseases that may not affect them until later life, as well as the potential social stigma. Different cultures may also view NBS in different lights which will often guide national policy that can differ greatly between countries. Taken together, these issues pose a serious challenge to implementing comprehensive NBS programs globally which ultimately impacts on our ability to identify and diagnose rare disease at an early stage.

Given IRDiRC's goal of shortening the diagnostic odyssey for RD patients, we have brought together international experts to provide insight into the current state of NBS worldwide, highlighting technological advances, as well as the many challenges, to implementing comprehensive screening programs.

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GENERAL INFORMATION

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

Virginie Bros-Facer[1,2], Stacie Taylor[3], Christine Patch[4]

[1]International Rare Diseases Research Consortium (IRDiRC), Hôpital Charles-Foix, Ivry-sur-Seine 94200, France.
[2]Medical Affairs Europe, Illumina, Évry-Courcouronnes 91000, France.
[3]Medical Affairs Global Scientific Communications, Illumina, San Diego, CA 92122, USA.
[4]Engagement and Society, Wellcome Connecting Science, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom.

Correspondence to: Dr Virginie Bros-Facer, Medical Affairs Europe, Illumina, 3 Rue Henri Auguste Desbruères, Évry-Courcouronnes, 91000, France. E-mail: vbros@illumina.com

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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

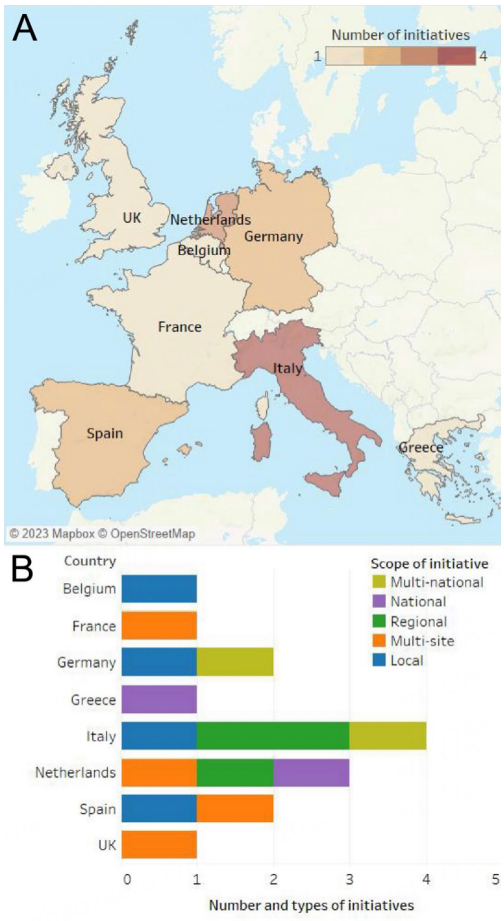

Figure 1. (A) shows a map of surveyed initiatives in Europe. The pan-European study is indicated twice as piloted in both Germany and Italy; (B) shows the scope of initiatives per country.

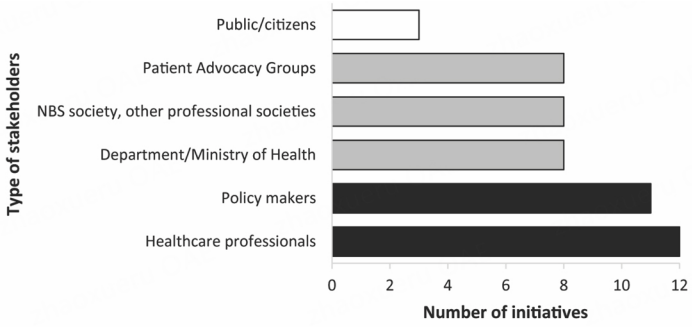

Figure 2. Desired impact on stakeholders.

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.

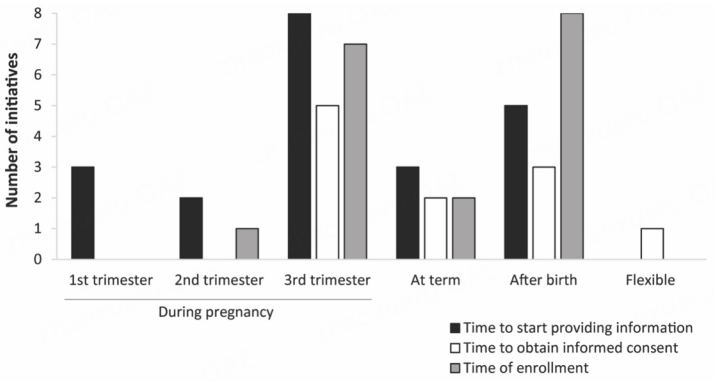

Figure 3. Timing for providing information, securing informed consent and enrollment.

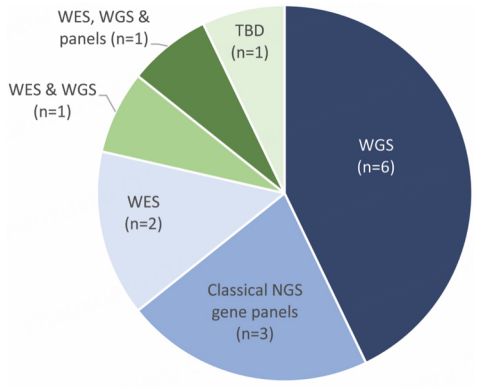

Figure 4. NGS approaches tested in the initiatives as a first-tier test for NBS.

• 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

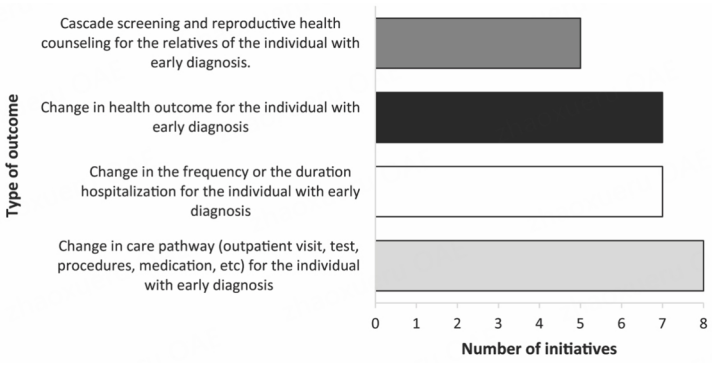

Figure 5. Outcome metrics that will be assessed during the follow-up.

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

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Not applicable.

Consent for publication

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Systematic Review

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

Giuditta Magnifico, Irene Artuso, Stefano Benvenuti

Fondazione Telethon ETS, Milan IT 20129, Italy.

Correspondence to: Dr. Stefano Benvenuti, Fondazione Telethon ETS, via Carlo Poerio 14, Milan IT20129, Italy. E-mail: sbenvenuti@telethon.it

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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.

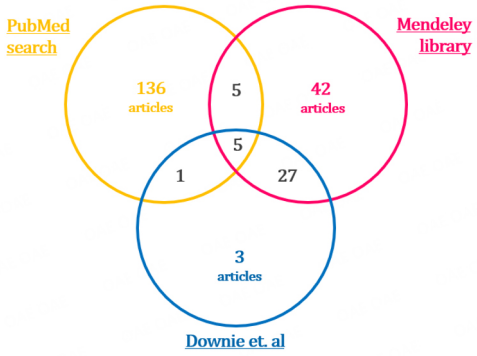

Figure 1. Venn diagram that shows the different sources of the articles.

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

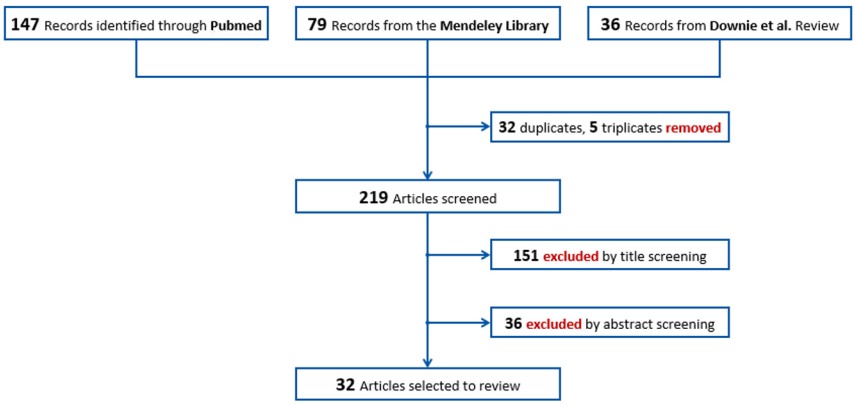

Figure 3. Study flowchart.

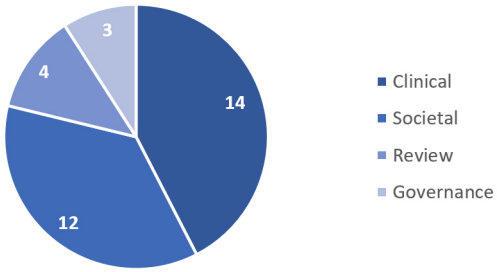

Figure 4. Distribution of the selected articles in the studied areas.

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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Review

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Development of newborn screening policies in Spain 2003-2022: what do we actually need to reach an agreement?

Cristina Valcárcel-Nazco[1,2,3,4], Lidia García-Pérez[1,2,3,4], Renata Linertová[1,2,3,4], Carmen Guirado-Fuentes[1,2,3,4], Aránzazu Hernández-Yumar[1,2,3], Lucinda Paz-Valiñas[3,5], Paula Cantero-Muñoz[3,5], Manuel Posada[6], Pedro Serrano-Aguilar[1,3]

[1]Servicio de Evaluación del Servicio Canario de la Salud (SESCS), Santa Cruz de Tenerife 38109, Spain.
[2]Fundación Canaria Instituto de Investigación Sanitaria de Canarias (FIISC), Las Palmas de Gran Canaria 35019 , Spain.
[3]Red Española de Agencias de Evaluación de Tecnologías Sanitarias y Prestaciones del Sistema Nacional de Salud (RedETS), Madrid 28071, Spain.
[4]Red de Investigación en Cronicidad, Atención Primaria y Prevención y Promoción de la Salud (RICAPPS), Madrid 28071, Spain.
[5]Unidade de Asesoramento Científico-técnico (Avalia-t). Axencia Galega de Coñecemento en Saúde (ACIS), Santiago de Compostela 15707, Spain.
[6]Instituto de Investigación en Enfermedades Raras (IIER), Instituto de Salud Carlos III (ISCIII), Madrid 28071, Spain.

Correspondence to: Cristina Valcárcel-Nazco, Servicio de Evaluación del Servicio Canario de la Salud (SESCS), Centro de Salud San Isidro-El Chorrillo, El Rosario, Santa Cruz de Tenerife 38109, Spain. E-mail: cristina.valcarcelnazco@sescs.es

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Abstract

Newborn screening (NBS) for inherited disorders is recognized as an essential public health intervention to improve health outcomes in the newborn population. The implementation of an NBS programme requires an evaluation of effectiveness, safety, cost-effectiveness, feasibility, and budget impact. Determining which of the known disorders should be included in NBS programmes is a public health policy challenge. In this context, economic evaluation aims to contribute to the sustainability of public health systems, but the appropriate economic evaluation framework for these interventions is still unclear. Existing NBS programmes vary widely in the number and type of disorders screened, even among the most developed European countries, despite the fact that the core criteria for guiding policy decision-making are standard. In Spain, where delivery of NBS programmes is marked by heterogeneity between regions, guidelines based on the best available scientific evidence are being established in order to achieve standardization of NBS policies and programmes at a national level. This paper

provides a general overview of existing evidence-based health-policy initiatives aimed at enhancing the equity and efficiency of the NBS programme in Spain and their impact on health decisions. We also describe existing challenges to reduce uncertainty, and the variations observed in decisions relating to the content and procedures used in NBS programmes.

Keywords: Newborn screening, inherited disorders, health-policy, equity

INCREASING INEQUALITIES IN NEWBORN SCREENING IN THE EUROPEAN UNION

NBS for inherited disorders is recognized as an essential public health intervention to improve health outcomes in the newborn population, by identifying and treating infants with life-threatening or debilitating disorders early in post-natal life, before clinical symptoms appear[1,2]. Determining which of the known disorders should be included in NBS programmes is a public health policy challenge that must take into account different perspectives (e.g., medicine, science, public policy, advocacy, ethics, and economics)[3]. The scientific evidence available to support decisions about which conditions should be included in NBS is often quantitatively and qualitatively limited, making it difficult to anticipate expected long-term outcomes that may occur, in order to support decision-making about extending NBS[4,5]. Policy makers and stakeholders must often rely on incomplete data and limited scientific evidence to decide whether a rapid expansion of the NBS panel is more desirable than maintaining a more deliberative pace[6,7]. Existing NBS programmes vary widely in the number and type of disorders screened[8-10], even among the most developed European countries, despite the fact that the core criteria for guiding policy decision-making are standard[11,12]. These criteria not only include an assessment of the effectiveness of the NBS but stress the need to assess economic consequences alongside other factors such as acceptance[11-13]. These policy variations and their consequences, such as health inequalities from the earlier stages of life, occur internationally and intra-nationally, regardless of epidemiological and socio-economic issues[14], especially in European countries such as Spain with decentralized governmental jurisdictions.

Past technological advances allowed for the expansion of NBS services[15-20], due mainly to the proven favourable performance and low costs of tandem mass spectrometry (MS/MS)[21,22], the development of new therapeutic options (e.g., gene therapy and enzyme replacement therapies), and improved knowledge of some inherited metabolic disorders[8,23]. Moreover, differences in the application and interpretation of universal screening implementation criteria at a health-policy level are increasing the variations observed in the contents of NBS programmes[9]. It is likely that with the increasing potential of next-generation genomic sequencing in NBS, these differences may become even more pronounced in the coming years[14], despite relevant uncertainties linked to classic Wilson and Jungner criteria, such as lack of knowledge about the natural history of many screened diseases, the potential harm of false-positive screening tests, the limited availability of effective treatments, or economic, social and ethical considerations[24]. Regardless of the existing frameworks for guiding health policy screening decisions[25], the subjective character of some criteria, such as the importance of the health problem in question, together with the accessibility of new, effective, and cost-effective screening methods, variations in the interpretation of available evidence for a given condition, and the public advocacy by families, professionals, and state legislators, have led to some of the observed variations[14].

Economic data relating to NBS have often been based on cost estimates rather than complete economic evaluations and opportunity-cost considerations[26,27]. Costs have played a favourable role in decisions because the direct costs of additional screening tests are relatively low, regardless of the considerable uncertainty about the effectiveness of available treatment options[26-29]. The role of costs needs to be

considered in depth and early on in the decision-making process. This is due to variations in treatment costs, hospital-billing policy, the requisite infrastructure (at all stages) and personnel cost of running the programme, as well as additional expertise and follow-up systems for surveillance. All of these aspects will affect the viability and sustainability of the NBS programme[6,30-33]. Aside from costs, additional unexplored variations in value judgments, driven by technological availability, social or professional pressure, or political opportunity, usually not explicitly stated, could play a differential role in policy decision-making[34].

There is no evidence to show that those populations who receive NBS for fewer disorders, such as in the UK, France, several Spanish regions, and Finland, are at a higher risk of poorer health outcomes than the populations of Austria, Italy, or Iceland, where NBS programmes cover more than 25 different conditions[3]. Moreover, governments are committed to ensuring the value of all health care provided to the population, as well as the long-term sustainability of the health care system per se. This commitment has recently been exacerbated by the double impact of the 2008 economic and financial crisis and the direct and collateral pandemic effects of COVID-19[35].

EVIDENCE-BASED HEALTH POLICY INITIATIVES TO ENHANCE EQUITY AND EFFICIENCY FOR THE NBS PROGRAMME IN SPAIN

The Spanish National Health Service (SNHS) is a decentralized public health insurance system with universal coverage, which provides free health care to every resident in all of the country's Autonomous Regions (ARs). The SNHS is managed at a regional level and fully financed by national insurance contributions and taxes, though some regional governments allocate local budgets to provide additional funding. The national government established three health policy instruments to support the decentralization of health planning and management competencies in each ARs: 1) the Spanish General Health Care Act, guaranteeing free and universal healthcare access to all Spanish residents[36]; 2) the National Benefits Catalogue to ensure equity in the supply of healthcare services[37]; and, 3) the Inter-territorial Board of the SNHS (made up of central and regional public health authorities). However, even though these health-policy instruments were established early to ensure equity, homogeneity, and efficiency in a decentralized SNHS, variations have since occurred in the supply of healthcare services, including NBS programmes[26].

Universal NBS, introduced in 1968 in Spain, is a well-established programme funded by every AR. It is currently organized through a network of 20 regional laboratories with an overall coverage of over 98% of the neonatal population. By 2000, the AR of Galicia had implemented a pioneering expanded NBS programme based on MS/MS for more than 40 conditions[23], at a time when the country's remaining ARs were mainly screening for phenylketonuria (PKU) and hypothyroidism. Despite the fact that the Galician programme was not expressly developed as a research initiative, it has nonetheless generated new knowledge about the true prevalence of a wide spectrum of disorders and relationships between biomarkers and disease expression in that region of Spain[38]. Subsequently, the SNHS Cohesion and Quality was operationalized by means of Quality Plans supported by the Spanish Network for Health Technology Assessment (RedETS), with the aim of bolstering evidence-based health policies and limiting variations in the supply of healthcare services among ARs[39]. As a result, there has been a growing demand for evidence and justification of value in the context of innovation and health technology assessment (HTA), whether to endorse funding, coverage, and reimbursement decisions or, alternatively, to support price negotiations[40].

To standardize the offer of NBS and reduce inequalities in the SNHS, the Spanish Ministry of Health prompted the creation of 1) a Framework Document to guide NBS decisions approved by the Public Health Commission of the SNHS Inter-territorial Board; and 2) an expert group to develop recommendations on i)

a uniform screening panel; ii) minimal standards for NBS in all ARs; iii) a decision matrix for potential NBS expansion; iv) a common quality-assurance process; and v) the development of a common information system for assessment, linked to the NBS. This expert group received scientific and technical support from RedETS through a series of HTA reports commissioned by the expert group, pursuant to an iterative consultative process involving national experts and AR health authorities. HTA reports delivered by RedETS provide appraised, evidence-based information on the safety, effectiveness, cost-effectiveness, legal, ethical, organizational, and environmental aspects of all non-pharmaceutical technologies[41].

COST-EFFECTIVENESS ISSUES IN NBS AND ITS DEVELOPMENT IN SPAIN

As previously reported, NBS cost estimates may be underestimated due to the relative inexpensiveness of adding "just one more test" to an existing screening panel. However, the cumulative cost of screening is beyond the test cost itself, in that it increases with the necessary acquisition of testing equipment, materials, plant, and staff[33]. According to the Hasting Reports[30], a health policy decision-making framework for NBS should be based on scientific evidence, in addition to 1) taking overall opportunity costs into account; 2) distributing the cost and benefits of the programme fairly; and 3) respecting human rights[24]. Beyond cost estimates of the potential incorporation of new disorders into NBS programmes, the need for cost-effectiveness analysis (CEA) results in the decision-making process is justified because the costs and health outcomes per screened newborn (comprising follow-up testing and subsequent lifelong treatment) are extended to all newborns at regional or national levels.

In 2012, Langer *et al.* drew up specific guidelines to assess and improve the methodological quality of economic evaluations of NBS. The authors assessed 12 CEA studies on NBS for inherited metabolic disorders by MS/MS, reporting differences in cost categories considered (from both health services and societal perspectives)[42]. When adopting the health services perspective, the costs should include those pertaining to the NBS programme as well as treatment and follow-up. The societal perspective should consider, in addition, the lost productivity of patients and informal caregivers[42]. Furthermore, one of the main problems confronting economic evaluations of NBS relates to the lack of epidemiological data and health outcomes (in terms of morbidity and mortality)[42]. In 2020, a scoping review on the challenges of economic evaluations of NBS showed that the methodological quality of these types of studies continues to be irregular[27].

In an economic evaluation, effectiveness is preferably measured by means of quality-adjusted life years (QALYs) gained. A QALY measures the health state of a person or group by adjusting the length of life to reflect the quality of life. The QALYs provide a common measure to compare the benefits gained from different alternative interventions. The threshold of willingness to pay for an additional QALY in Spain has been estimated at around €25,000/QALY[43]. However, the low frequency of these disorders and the methodological complexities of estimating QALYs justify the fact that life years (LY) gained are used as an alternative outcome measure[42,44].

The validity and robustness of CEA estimates are limited by the uncertainty of relevant parameters (incidence of disorders, short-term screening effectiveness, and the long-term consequences of screening). Addressing this uncertainty requires the use of simulation modelling techniques[42,44] and corresponding sensitivity analyses that depend on arguable assumptions. Logistic and ethical considerations lead to the absence of clinical trials on NBS, making it necessary to use effectiveness data from case series, mainly short-term, very small, and subject to biases. Well-structured and completed population-based registries, which include the necessary long-term follow-up of cases as well as comprehensive information on the natural history of diseases, would seem a promising tool to improve the availability and validity of data.

NBS's impact on health service utilization and health outcomes might be lifelong. However, the effect of screening on early identification of conditions is mainly based on extrapolated short-term outcomes and data quantification in adulthood is inadequate. Consequently, CEAs are restricted to short timeframes based on the available information or, alternatively, incorporate an appropriate time horizon relying on assumptions. Specific aspects such as incidence, prevalence and costs are likely to be different between countries, not only for NBS itself, but also for all subsequent treatment and follow-up activities. Therefore, the transferability to different contexts of CEAs on NBS is limited and requires review, revision, and adaptation on the basis of local data[44].

Since 2006, an HTA perspective, including CEA, has been required by the Spanish Ministry of Health for decision-making on the potential extension of the national NBS programme (available from[39]). Unfortunately, as epidemiological, clinical, and economic data relating to NBS are in short supply and unreliable, CEA studies are limited. From 2006 to 2022, RedETS provided evidence of the effectiveness and cost-effectiveness of an expanded NBS programme at the national level. RedETS drew up CEAs comparing costs and outcomes for 16 different disorders from both societal and national health system perspectives. The first CEA report carried out in 2006 determined the cost-effectiveness of MS/MS to screen PKU and medium chain 3-hydroxyacyl-CoA dehydrogenase deficiency (MCADD) in Spain, with an incremental cost-effectiveness ratio (ICER) below €6000/LY[22]. Following this report, RedETS estimated the ICER of adding five new disorders to the NBS national programme (based on MS/MS for PKU and MCADD): homocystinuria, long chain 3-hydroxyacyl- CoA dehydrogenase deficiency (LCHADD), maple syrup urine disease, isovaleric acidaemia and glutaric aciduria type 1 (GA-I)[44]. The incorporation of this set of diseases yielded an ICER of €28,000/LY approximately. Subsequently, other reports were prepared in which the cost-effectiveness of neonatal screening for different diseases, not all of which subject to MS/MS, was estimated: sickle cell disease, cystic fibrosis, biotinidase deficiency, congenital adrenal hyperplasia, galactosaemia, methylmalonic and propionic acidaemia, tyrosinaemia type I, primary carnitine deficiency (CUD), very long-chain acyl-CoA dehydrogenase deficiency (VLCAD), and severe combined immunodeficiency (SCID) (available from[39]). It should be noted that the results of these reports showed that adding propionic and methylmalonic acidaemia, VLCAD, and CUD to the national NBS programme is a cost-effective alternative in Spain (ICER: €21,405/QALY, €10,723/LY and €14,217/LY, respectively). In sharp contrast, the ICER for tyrosinaemia type 1 was €30,034/QALY. In addition, other diseases whose screening was shown to be a cost-effective technology were biotinidase, congenital adrenal hyperplasia, or cystic fibrosis (all of them yielded ICERs of below €30,000/LY). Finally, sickle cell disease screening would be cost-effective if the cost per newborn screened did not exceed €2.5 and SCID screening would be below the Spanish cost-effectiveness threshold of €25,000 per QALY[43] as long as the cost of the screening test does not exceed €4 per newborn[39].

From the evidence generated in this set of HTA reports, a set of seven disorders were included in the national NBS programme in 2014 (hypothyroidism, PKU, cystic fibrosis, sickle cell disease, MCADD, LCHADD, and GA-I[44]) and four additional diseases were added in 2022 (biotinidase deficiency, maple syrup urine disease, homocystinuria, and congenital adrenal hyperplasia[39]). The Spanish NBS programme could be expanded in the near future, depending upon new scientific evidence, the development of new detection biomarkers, enhanced knowledge of the natural history, and new therapies that may possibly emerge for the respective disorders.

STAKEHOLDER PARTICIPATION TO SUPPORT DECISION-MAKING ON NEWBORN SCREENING IN SPAIN

Beyond cost-effectiveness, several other previously mentioned factors influence policy-making in NBS. Differences in the interpretation and management of uncertainties in these factors might explain observed

variations in the content of NBS programmes at a regional/national level[16,45]. Moreover, as the number of disorders added to NBS programmes increases, maintaining a balance between privacy and the rights of society (parents), a debate arises as to the use of screening in cases where the reliability and availability of treatment are limited. Hence, to explore the "benefit potentials" of screening, RedETS involved patient associations, expert physicians, scientific societies, and public health professionals along the HTA process, in order to inform policy decisions.

The relatives of affected people, integrated into officially constituted patient associations, were deliberately involved from the beginning of the evaluation process of each of the selected disorders so that they could make their viewpoint known and make a contribution to relevant aspects[46-48]. In addition, a call to participate was made to the major patient federations that bring together most patient associations, such as the Patient Platform, the Spanish Patient Forum, the Spanish Federation of Rare Diseases, and the Spanish Federation of Metabolic Diseases. Designated representatives were included as collaborating experts in the teams that laid the foundations for drawing up both the protocol and the assessment report.

Scientific societies linked either to the technology to be assessed or to the health problem targeted, such as the Spanish Association for the Study of Inborn Errors of Metabolism (AECOM), Spanish Paediatrics Association (AEP), Spanish Society of Inborn Errors of Metabolism (SEEIM), Spanish Society of Laboratory Medicine, Perinatal Diagnosis Commission (SEQCML-DP), Spanish Society of Epidemiology (SEE), Rare Diseases Research Institute (IIER), and the Federation of Spanish Medical Scientific Associations, were also invited to take part as experts in the drafting of HTA reports. Additionally, individual experts were sought through an informal review of indexed scientific publications related to the subject, and invited to participate in the preparation of the report. Similarly, industry representatives were invited to participate in the assessment process from its inception, undertake protocol data verification and provide additional information of interest. In the end, they were able to review the final report and make amendments to it.

IMPACT OF EVIDENCE-BASED HEALTH POLICY DECISION-MAKING FOR NBS IN SPAIN

Table 1 shows changes in the number of neonatal disorders included in regional NBS programmes in Spain between 2003 and 2020. As can be seen, there was an increase in regional variations, despite the SNHS Inter-territorial Board's recommendations to the ARs, made by the experts and based on the availability of a wide series of HTA reports issued by RedETS. Although a group of six ARs accepted the Ministry of Health's recommendations, offering evidence-based NBS programmes limited to seven disorders of proven effectiveness and cost-effectiveness, other ARs offer programmes in which the number of screened disorders ranges from 18 to 40.

WHAT WE HAVE LEARNT

As shown, the Spanish Ministry of Health considers evidence-based data essential to inform decisions on NBS programme revisions. Therefore, considering the natural history and consequences of a given disorder, the accuracy of screening for it, the harms and benefits of early diagnosis[2], and the opportunity cost of publicly funding it are key elements in the decision-making process[39,44]. Unfortunately, in Spain and other countries, common NBS programme policy decisions are often made with limited evidence and incomplete data. Decisions around these public health programmes become challenging when disorders are rare with a low incidence, advocates have a stronger role in decisions, new treatments are emerging, and technology makes NBS more feasible[49], sometimes with undesirable results[50].

Table 1. Number of neonatal disorders included in regional NBS programmes: Spain 2003 and 2020

Spain	2003* Without availability of HTA reports	2020 Supported by HTA reports
Castilla-León	3	7
Asturias	3	7
Baleares	3	7
Canarias	2	7
Cantabria	2	7
Navarra	2	7
Valencia	3	7
Pais Vasco	2	7
Extremadura	5	18
Madrid	3	18
Castilla La Mancha	4	22
Cataluña	3	25
Aragón	3	30
La Rioja	3	30
Galicia	25	30
Andalucía	3	35
Murcia	3	40

*Data should be taken with caution, given the lack of validated records from 2003.

This paper shows that variations in the content of NBS programmes can actually increase, notwithstanding the existence of a specific procedure to guide decisions based on the critical evaluation of the best available scientific evidence of such programmes' effectiveness, cost-effectiveness, and economic, organizational, ethical, and social impacts. Although the data reported here refer to different Spanish regions, similar findings are observed in other developed countries worldwide[10,51]. It is possible that some of the differences observed among countries could be accounted for by the different considerations of economic aspects in general, and, particularly, the availability and consideration of economic evaluations adapted to each country[10,52]. While in some cases, there is no information available on the cost-effectiveness of the screening programmes under consideration, in others, this information is not valued or used, regardless of whether it might be available[53,54]. It is somewhat more difficult to interpret the differences observed between the Spanish ARs, which seem to fall into two blocs, one that clearly adheres to recommendations based on cost-effectiveness criteria, and the other, in which application of this criterion might be diluted by the effect of other potential considerations.

Economic evaluation, in terms of cost-effectiveness or cost-utility, is a central component of health technology assessment reports to inform public funding decisions for new interventions, given that budgets do not grow at the speed at which healthcare innovations of potential value do. Precisely to ensure that available budgets are allocated to fund innovations of the greatest value to health, CEAs combine costs and health outcomes and compare them with those corresponding to other innovations, possibly even outside the scope of NBS programmes. This procedure brings transparency and reproducibility to decision-making. It is true, however, that this procedure might stop funding emerging for diagnostic or therapeutic innovations aimed at people affected by rare diseases, for which there are few alternatives, either because of the relative lack of robust evidence of their efficacy or their high cost. In order to address the barrier posed by the cost of innovations in the field of rare diseases, without sacrificing transparency and reproducibility in decision-making, one proposal is to establish threshold values of willingness to pay other than those of the general population[55,56]. Another procedure increasingly used to guide innovation-funding decisions

uses Multicriteria Decision Analysis to ensure that all relevant criteria, including societal values, are considered in a more open and participatory manner, in which costs or cost-effectiveness data do not constitute a limiting barrier[57,58].

In this scenario of searching for procedures to inform health policy decisions in a manner that is responsible, transparent, reproducible, participatory, and sensitive to the unmet needs of society, it is still necessary to intensify efforts to obtain more valid information on effectiveness by making available information on relevant health outcomes. Sharing research information and promoting pilot studies among international partners is a vital part of the process of expanding NBS, by developing consistent case definitions for conditions included within NBS programmes, helping to ensure the interoperability of long-term outcome studies to increase the availability of screening, and subsequently benefiting wider populations[24]. Opportunity costs and potential benefits beyond the child should be factored in, thus structuring a policy that will distribute costs and benefits fairly[30].

Regional studies using long-term observational designs have been set in motion in some countries to evaluate the feasibility, diagnostic-process quality, and population distribution of biomarkers, by defining cut-off values, establishing patient-care pathways and algorithms for patient care, and measuring longer-term relevant health benefits[58-60]. Furthermore, national registries for inborn errors of metabolism have been implemented to assess outcomes at a population level, while also acting as electronic medical records that help clinicians monitor treatment and record progress, as in Sweden[61]. To address substantial gaps in NBS evidence, the US State of North Carolina established the Early Check initiative as a translational research enterprise embedded in a public health programme, with the dual aim of informing NBS policy and quantifying the potential benefits and risks of early identification and pre-symptomatic treatment of infants that have rare disorders, for a select number of conditions, offered as a supplement to standard NBS to all birthing parents under a voluntary research protocol[2]. Similarly, the Spanish Ministry of Health has developed Post-Launch Evidence Generation (PLEG) studies, which can provide post-marketing real-world evidence, thereby enlarging the scope of HTA in the life cycle of innovations[62] while making the innovation under research accessible to society. The PLEG studies can be boosted at the request of health authorities, because of their need to rely on additional evidence to make decisions about innovations with high potential value but limited knowledge regarding their long-term effects. Despite the interest in these regional/national initiatives, as the COVID-19 pandemic has recently revealed, more international collaboration within the European Union is required to increase sample sizes and reduce the uncertainty of the different parameters of NBS programmes, something that translates as substantial variations in the number of disorders screened and, probably, in the quality of their application and the results obtained in the short, medium, and long term.

It is possible, however, that all these efforts to reduce uncertainty and variations in decisions relating to the content and procedures used in NBS programmes would not suffice to resolve the inequalities in the supply of services between territories, as shown in this paper. It is also necessary to know and understand both the decision-making processes and the criteria applied by the decision-makers in the different Spanish regions, which have resulted in such different decisions, after starting from agreed political principles and a set of common data based on scientific evidence. Accordingly, qualitative research must be incorporated into these aspects, covering all the actors involved in decision-making.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the study, performed data interpretation, revised the draft critically for important intellectual content and finally approved the version to be

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Performed data acquisition and interpretation, as well as providing technical and material support and revising the draft critically: Renata Linertová R, Guirado-Fuentes C, Hernández-Yumar A, Paz-Valiñas L, Cantero-Muñoz P

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Rare Disease and Orphan Drugs Journal

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?

Petros Tsipouras[1], Maria Chatzou Dunford[2], Hadley Sheppard[2], Hannah Gaimster[2], Theoklis Zaoutis[3,4]

[1]FirstSteps Greece, Newborn genome screening Initiative, Athens 106 80, Greece.
[2]Lifebit Biotech Ltd., London EC2A 2AP, United Kingdom.
[3]National Public Health Organization (EODY), Athens 151 23, Greece.
[4]The 2nd Department of Pediatrics, National and Kapodistrian University of Athens, 'P. & A. Kyriakou' Children's Hospital, Athens 106 80, Greece.

Correspondence to: Dr. Petros Tsipouras, FirstSteps Greece, Newborn genome screening Initiative, Skoufa 64, Athens 106 80, Greece. E-mail: petros.tsipouras@beginnings.gr

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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

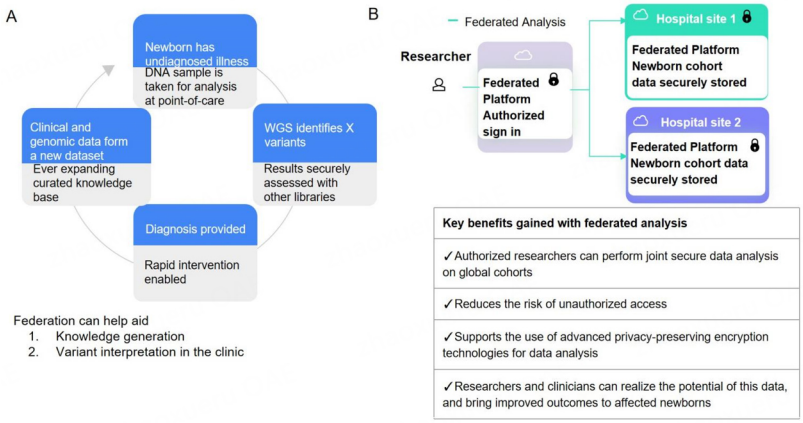

Figure 1. An overview of how federated data analysis can be incorporated into an undiagnosed disease program targeting newborns to help enable secure data access across research laboratories and clinics worldwide. (A) The steps involved in diagnosing a rare disease in an affected newborn; (B) A summary 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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Not applicable.

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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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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.

If needed, authors are recommended to consider the language editing services provided by Charlesworth to ensure that the manuscript is written in correct scientific English before submission. Authors who publish with OAE journals enjoy a special discount for the services of Charlesworth via the following two ways.

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1.5 Work Funded by the National Institutes of Health

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.

2. Submission Preparation

2.1 Cover Letter

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, Case Report, *etc.*) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;

In the second paragraph: concisely explain what was done, the main findings and why they are significant;

In the third paragraph: indicate why the manuscript fits the Aims and Scope of the journal, and why it would be attractive to readers;

In the fourth paragraph: confirm that the manuscript has not been published elsewhere and not under consideration of any other journal. All authors have approved the manuscript and agreed on its submission to the journal. Journal’s specific requirements have been met if any.

If the manuscript is contributed to a special issue, please also mention it in the cover letter.

If the manuscript was presented partly or entirely in a conference, the author should clearly state the background information of the event, including the conference name, time and place in the cover letter.

2.2 Types of Manuscripts

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	Abstract	Keywords	Main Text Structure
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	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 paper summarizes the literature on previous studies. It usually does not present any new information on a subject.	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.
Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
Meta-Analysis	A Meta-Analysis is a statistical analysis combining the results of multiple scientific studies. It is often an overview of clinical trials.	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.	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.
Technical Note	A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applied in research.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Editorial	An Editorial is a short article describing news about the journal or opinions of senior editors or the publisher.	None required	None required	/
Letter to Editor	A Letter to Editor is usually an open post-publication review of a paper from its readers, often critical of some aspect of a published paper. Controversial papers often attract numerous Letters to Editor	Unstructured abstract (optional). No more than 250 words.	3-8 keywords (optional)	/
Opinion	An Opinion usually presents personal thoughts, beliefs, or feelings on a topic.	Unstructured abstract (optional). No more than 250 words.	3-8 keywords	/
Perspective	A Perspective provides personal points of view on the state-of-the-art of a specific area of knowledge and its future prospects. Links to areas of intense current research focus can also be made. The emphasis should be on a personal assessment rather than a comprehensive, critical review. However, comments should be put into the context of existing literature. Perspectives are usually invited by the Editors.	Unstructured abstract. No more than 150 words.	3-8 keywords	/

2.3 Manuscript Structure

2.3.1 Front Matter

2.3.1.1 Title

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.

2.3.1.2 Authors and Affiliations

Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

2.3.1.3 Abstract

The abstract should be a single paragraph with word limitation and specific structure requirements (for more details please refer to Types of Manuscripts). It usually describes the main objective(s) of the study, explains how the study was done, including any model organisms used, without methodological detail, and summarizes the most important results and their significance. The abstract must be an objective representation of the study: 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.

2.3.1.4 Keywords

Three to eight keywords should be provided, which are specific to the article, yet reasonably common within the subject discipline.

2.3.2 Main Text

Manuscripts of different types are structured with different sections of content. Please refer to Types of Manuscripts to make sure which sections should be included in the manuscripts.

2.3.2.1 Introduction

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 the aim was achieved. Relevant controversies or disagreements in the field should be introduced as well.

2.3.2.2 Methods

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.

2.3.2.3 Results

This section contains the findings of the study. 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 Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study. Future research directions may also be mentioned.

2.3.2.5 Conclusion

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 acknowledge.

2.3.3.2 Authors' Contributions

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.

Please use Surname and Initial of Forename to refer to an author's contribution. For example: made substantial contributions to conception and design of the study and performed data analysis and interpretation: Salas H, Castaneda WV; performed data acquisition, as well as provided administrative, technical, and material support: Castillo N, Young V.

If an article is single-authored, please include "The author contributed solely to the article." in this section.

2.3.3.3 Availability of Data and Materials

In order to maintain the integrity, transparency and reproducibility of research records, authors should include this section in their manuscripts, detailing where the data supporting their findings can be found. Data can be deposited into data repositories or published as supplementary information in the journal. Authors who cannot share their data should state that the data will not be shared and explain it. If a manuscript does not involve such issue, please state "Not applicable." in this section.

2.3.3.4 Financial Support and Sponsorship

All sources of funding for the study reported should be declared. The role of the funding body in the experiment design, collection, analysis and interpretation of data, and writing of the manuscript should be declared. Any relevant grant numbers and the link of funder's website should be provided if any. If the study is not involved with this issue, state "None." in this section.

2.3.3.5 Conflicts of Interest

Authors must declare any potential conflicts of interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there are no conflicts of interest, please state "All authors declared that there are no conflicts of interest." in this section. Some authors may be bound by confidentiality agreements. In such cases, in place of itemized disclosures, we will require authors to state "All authors declare that they are bound by confidentiality agreements that prevent them from disclosing their conflicts of interest in this work.". If authors are unsure whether conflicts of interest exist, please refer to the "Conflicts of Interest" of *RDODJ* Editorial Policies for a full explanation.

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Research involving human subjects, human material or human data must be performed in accordance with the Declaration of Helsinki and approved by an appropriate ethics committee. An informed consent to participate in the study should also be obtained from participants, or their parents or legal guardians for children under 16. A statement detailing the name of the ethics committee (including the reference number where appropriate) and the informed consent obtained must appear in the manuscripts reporting such research.

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2.3.3.7 Consent for Publication

Manuscripts containing individual details, images or videos, must obtain consent for publication from that person, or in the case of children, their parents or legal guardians. If the person has died, consent for publication must be obtained from the next of kin of the participant. Manuscripts must include a statement that a written informed consent for publication was obtained. Authors do not have to submit such content accompanying the manuscript. However, these documents must be available if requested. If the manuscript does not involve this issue, state "Not applicable." in this section.

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2.3.3.9 References

Preferably original research articles that directly support the statements should be cited. Review articles could be cited when they specifically address the statement made in the manuscript. An abstract should not be used as a reference. Non-specific citations should be avoided.

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.

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.

For other types of references, please refer to U.S. National Library of Medicine.
The journal also recommends that authors prepare references with a bibliography software package, such as EndNote to avoid typing mistakes and duplicated references.

2.3.3.10 Supplementary Materials

Additional data and information can be uploaded as Supplementary Materials to accompany the manuscripts. The supplementary materials will also be available to the referees as part of the peer-review process. Any file format is acceptable, such as data sheet (word, excel, csv, cdx, fasta, pdf or zip files), presentation (powerpoint, pdf or zip files), image (cdx, eps, jpeg, pdf, png or tiff), table (word, excel, csv or pdf), audio (mp3, wav or wma) or video (avi, divx, flv, mov, mp4, mpeg, mpg or wmv). All information should be clearly presented. Supplementary materials should be cited in the main text in numeric order (e.g., Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2, *etc.*). The style of supplementary figures or tables complies with the same requirements on figures or tables in main text. Videos and audios should be prepared in English and limited to a size of 500 MB.

2.4 Manuscript Format

2.4.1 File Format

Manuscript files can be in DOC and DOCX formats and should not be locked or protected.

2.4.2 Length

There are no restrictions on paper length, number of figures, or amount of supporting documents. Authors are encouraged to present and discuss their findings concisely.

2.4.3 Language

Manuscripts must be written in English.

2.4.4 Multimedia Files

The journal supports manuscripts with multimedia files. The requirements are listed as follows:
Videos or audio files are only acceptable in English. The presentation and introduction should be easy to understand. The frames should be clear, and the speech speed should be moderate.
A brief overview of the video or audio files should be given in the manuscript text.
The video or audio files should be limited to a size of up to 500 MB.
Please use professional software to produce high-quality video files, to facilitate acceptance and publication along with the

submitted article. Upload the videos in mp4, wmv, or rm format (preferably mp4) and audio files in mp3 or wav format.

2.4.5 Figures

Figures should be cited in numeric order (e.g., Figure 1, Figure 2) and placed after the paragraph where it is first cited;
Figures can be submitted in format of tiff, psd, AI or jpeg, with resolution of 300-600 dpi;
Figure caption is placed under the Figure;
Diagrams with describing words (including, flow chart, coordinate diagram, bar chart, line chart, and scatter diagram, *etc.*) should be editable in word, excel or powerpoint format. Non-English information should be avoided;
Labels, numbers, letters, arrows, and symbols in figure should be clear, of uniform size, and contrast with the background;
Symbols, arrows, numbers, or letters used to identify parts of the illustrations must be identified and explained in the legend;
Internal scale (magnification) should be explained and the staining method in photomicrographs should be identified;
All non-standard abbreviations should be explained in the legend;
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2.4.6 Tables

Tables should be cited in numeric order and placed after the paragraph where it is first cited;
The table caption should be placed above the table and labeled sequentially (e.g., Table 1, Table 2);
Tables should be provided in editable form like DOC or DOCX format (picture is not allowed);
Abbreviations and symbols used in table should be explained in footnote;
Explanatory matter should also be placed in footnotes;
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2.4.7 Abbreviations

Abbreviations should be defined upon first appearance in the abstract, main text, and in figure or table captions and used consistently thereafter. Non-standard abbreviations are not allowed unless they appear at least three times in the text. Commonly-used abbreviations, such as DNA, RNA, ATP, *etc*., can be used directly without definition. Abbreviations in titles and keywords should be avoided, except for the ones which are widely used.

2.4.8 Italics

General italic words like *vs*., *et al., etc*., *in vivo*, *in vitro*; *t* test, *F* test, *U* test; related coefficient as *r*, sample number as *n*, and probability as *P*; names of genes; names of bacteria and biology species in Latin.

2.4.9 Units

SI Units should be used. Imperial, US customary and other units should be converted to SI units whenever possible. There is a space between the number and the unit (i.e., 23 mL). Hour, minute, second should be written as h, min, s.

2.4.10 Numbers

Numbers appearing at the beginning of sentences should be expressed in English. When there are two or more numbers in a paragraph, they should be expressed as Arabic numerals; when there is only one number in a paragraph, number < 10 should be expressed in English and number > 10 should be expressed as Arabic numerals. 12345678 should be written as 12,345,678.

2.4.11 Equations

Equations should be editable and not appear in a picture format. Authors are advised to use either the Microsoft Equation Editor or the MathType for display and inline equations.

2.5 Submission Link

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