Bros-Facer *et al. Rare Dis Orphan Drugs J* 2023;2:21
DOI: 10.20517/rdodj.2023.26

Rare Disease and Orphan Drugs Journal

Original Article

Open Access

Check for updates

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

How to cite this article: Bros-Facer V, Taylor S, Patch C. Next-generation sequencing-based newborn screening initiatives in Europe: an overview. *Rare Dis Orphan Drugs J* 2023;2:21. https://dx.doi.org/10.20517/rdodj.2023.26

Received: 8 Aug 2023 **First Decision:** 8 Sep 2023 **Revised:** 20 Sep 2023 **Accepted:** 26 Sep 2023 **Published:** 7 Oct 2023

Academic Editor: Daniel Scherman **Copy Editor:** Dan Zhang **Production Editor:** Dan Zhang

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

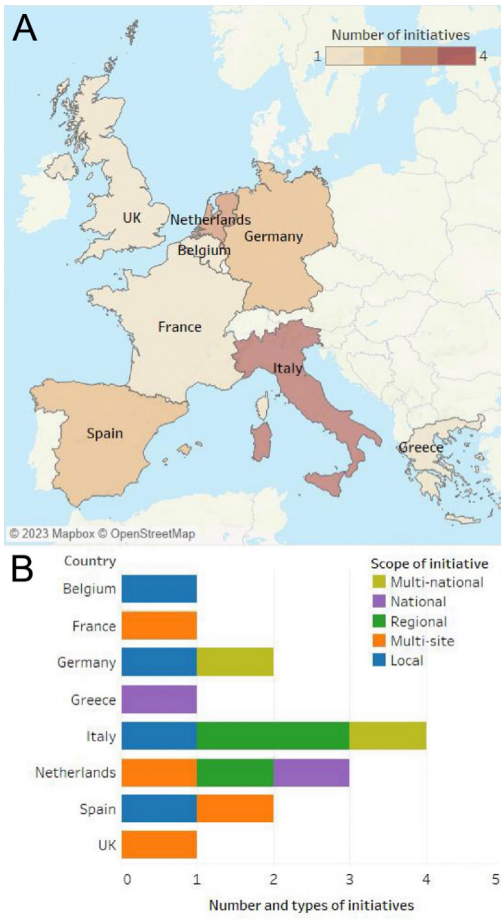

Figure 1. (A) shows a map of surveyed initiatives in Europe. The pan-European study is indicated twice as piloted in both Germany and Italy; (B) shows the scope of initiatives per country.

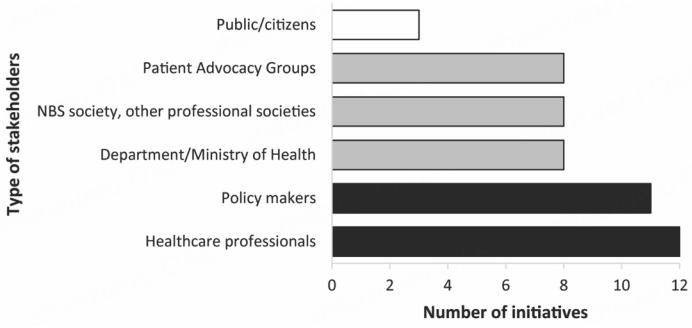

Figure 2. Desired impact on stakeholders.

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.

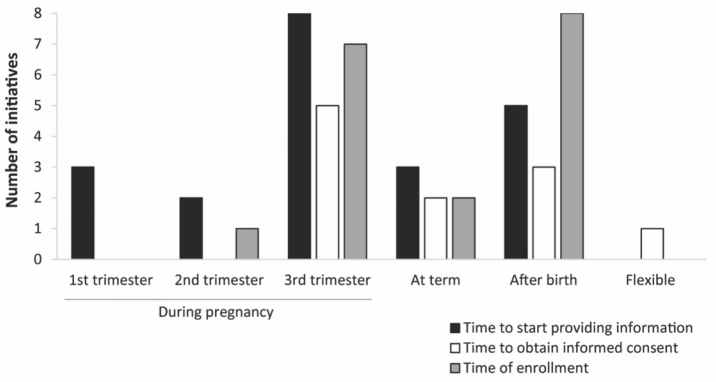

Figure 3. Timing for providing information, securing informed consent and enrollment.

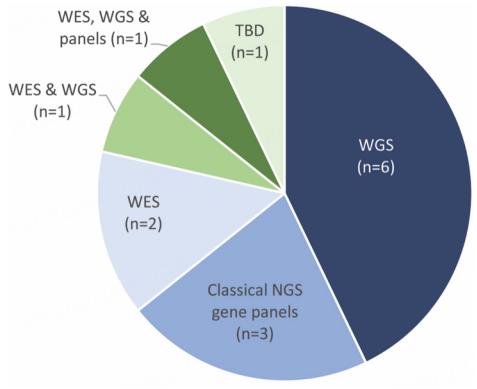

Figure 4. NGS approaches tested in the initiatives as a first-tier test for NBS.

• 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

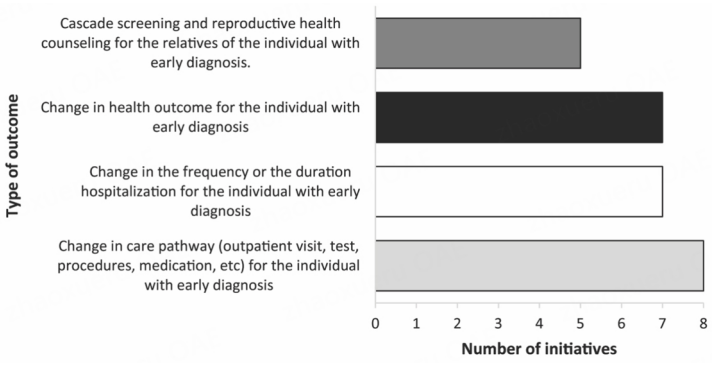

Figure 5. Outcome metrics that will be assessed during the follow-up.

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.

DECLARATIONS

Acknowledgments

The authors would like to thank all respondents to the survey and their colleagues involved in the initiatives, including Angel Carracedo, Maria Luce Couce Pico and Maria Eugenia Vazquez-Mosquera; Giorgio Casari; Laurence Faivre; Alessandra Ferlini and Nicolas Garnier; Mattia Gentile; Maria Iascone; Belen Perez and Francesc Palau; Amanda Pichini; Birgit Raddatz and Francjan van Spronsen; Wendy Rodenburg and Els Voorhoeve; Christian Schaaf, Maja Hempel and Heiko Brennenstuhl; Laurent Servais; Petros Tsipouras and Mirjam van der Burg. We would also like to thank Maria Martinez-Fresno for her help drafting the questionnaire, Raye Alford for reviewing and revising the manuscript for grammar and syntax, and Kirsten Curnow for her help with the figures.

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.

Financial support and sponsorship

Not applicable.

Conflicts of interest

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

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Zytkovicz TH, Fitzgerald EF, Marsden D, et al. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;47:1945-55. DOI PubMed
2. Chace DH, Spitzer AR. Altered metabolism and newborn screening using tandem mass spectrometry: lessons learned from the bench to bedside. *Curr Pharm Biotechnol* 2011;12:965-75. DOI PubMed
3. Watson MS, Lloyd-Puryear MA, Howell RR. The progress and future of US newborn screening. *Int J Neonatal Screen* 2022;8:41. DOI PubMed PMC
4. van Karnebeek CD, Tiebout SA, Niermeijer J, et al. Pyridoxine-dependent epilepsy: an expanding clinical spectrum. *Pediatr Neurol* 2016;59:6-12. DOI
5. McCombie WR, McPherson JD, Mardis ER. Next-generation sequencing technologies. *Cold Spring Harb Perspect Med* 2019;9:a036798. DOI PubMed PMC
6. Dimmock D, Caylor S, Waldman B, et al. Project baby bear: rapid precision care incorporating rWGS in 5 California children's hospitals demonstrates improved clinical outcomes and reduced costs of care. *Am J Hum Genet* 2021;108:1231-8. DOI PubMed PMC

7. Krantz ID, Medne L, Weatherly JM, et al; NICUSeq Study Group. Effect of whole-genome sequencing on the clinical management of acutely Ill infants with suspected genetic disease: a randomized clinical trial. *JAMA Pediatr* 2021;175:1218-26. DOI PubMed PMC
8. Petrikin JE, Cakici JA, Clark MM, et al. The NSIGHT1-randomized controlled trial: rapid whole-genome sequencing for accelerated etiologic diagnosis in critically Ill infants. *NPJ Genom Med* 2018;3:6. DOI
9. Farnaes L, Hildreth A, Sweeney NM, et al. Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. *NPJ Genom Med* 2018;3:10. DOI PubMed PMC
10. Mestek-Boukhibar L, Clement E, Jones WD, et al. Rapid paediatric sequencing (RaPS): comprehensive real-life workflow for rapid diagnosis of critically ill children. *J Med Genet* 2018;55:721-8. DOI PubMed PMC
11. van Diemen CC, Kerstjens-Frederikse WS, Bergman KA, et al. Rapid targeted genomics in critically Ill newborns. *Pediatrics* 2017;140:e20162854. DOI
12. Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. *Lancet Respir Med* 2015;3:377-87. DOI PubMed PMC
13. Ceyhan-Birsoy O, Murry JB, Machini K, et al; BabySeq Project Team. Interpretation of genomic sequencing results in healthy and Ill newborns: results from the BabySeq project. *Am J Hum Genet* 2019;104:76-93. DOI
14. Dimmock DP, Clark MM, Gaughran M, et al; RCIGM Investigators. An RCT of rapid genomic sequencing among seriously Ill infants results in high clinical utility, changes in management, and low perceived harm. *Am J Hum Genet* 2020;107:942-52. DOI PubMed PMC
15. Bick D, Bick SL, Dimmock DP, Fowler TA, Caulfield MJ, Scott RH. An online compendium of treatable genetic disorders. *Am J Med Genet C Semin Med Genet* 2021;187:48-54. DOI PubMed PMC
16. Owen MJ, Lefebvre S, Hansen C, et al. An automated 13.5 hour system for scalable diagnosis and acute management guidance for genetic diseases. *Nat Commun* 2022;13:4057. DOI PubMed PMC
17. GUARDIAN Study. Available from: https://guardian-study.org [Last accessed on 27 Sep 2023].
18. BeginNGS. Available from: https://radygenomics.org/begin-ngs-newborn-sequencing/ [Last accessed on Last accessed on 27 Sep 2023].
19. Early Check. Available from: https://earlycheck.org/news-and-outreach/newsroom/ [Last accessed on 27 Sep 2023].
20. Screen4Care (European Union). Available from: https://screen4care.eu/ [Last accessed on 27 Sep 2023].
21. Pichini A, Ahmed A, Patch C, et al. Developing a national newborn genomes program: an approach driven by ethics, engagement and co-design. *Front Genet* 2022;13:866168. DOI PubMed PMC
22. The UK Newborn Genomes Programme. Available from: https://www.genomicsengland.co.uk/initiatives/newborns [Last accessed on 27 Sep 2023].
23. Baby Detect. Available from: https://babydetect.com [Last accessed on 27 Sep 2023].
24. Stark Z, Scott RH. Genomic newborn screening for rare diseases. *Nat Rev Genet* 2023;24:755-66. DOI PubMed
25. Wilson JMG, Jungner G; World Health Organization. Principles and practice of screening for disease. Geneva: World Health Organization; 1968. Available from: https://policycommons.net/artifacts/537214/principles-and-practice-of-screening-for-disease-j/1513770/ [Last accessed on 27 Sep 2023].
26. Key principles for newborn screening (2021). Available from: https://www.eurordis.org/publications/key-principles-for-newborn-screening/ [Last accessed on 27 Sep 2023].
27. Balciuniene J, Liu R, Bean L, et al. At-risk genomic findings for pediatric-onset disorders from genome sequencing vs medically actionable gene panel in proactive screening of newborns and children. *JAMA Netw Open* 2023;6:e2326445. DOI PubMed PMC
28. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-24. DOI PubMed PMC
29. Oliver GR, Hart SN, Klee EW. Bioinformatics for clinical next generation sequencing. *Clin Chem* 2015;61:124-35. DOI PubMed
30. Owen MJ, Niemi AK, Dimmock DP, et al. Rapid sequencing-based diagnosis of thiamine metabolism dysfunction syndrome. *N Engl J Med* 2021;384:2159-61. DOI PubMed PMC
31. Austin-Tse CA, Jobanputra V, Perry DL, et al; Medical Genome Initiative*. Best practices for the interpretation and reporting of clinical whole genome sequencing. *NPJ Genom Med* 2022;7:27. DOI PubMed PMC
32. Souche E, Beltran S, Brosens E, et al. Recommendations for whole genome sequencing in diagnostics for rare diseases. *Eur J Hum Genet* 2022;30:1017-21. DOI PubMed PMC
33. Nurchis MC, Riccardi MT, Radio FC, et al. Incremental net benefit of whole genome sequencing for newborns and children with suspected genetic disorders: systematic review and meta-analysis of cost-effectiveness evidence. *Health Policy* 2022;126:337-45. DOI
34. NIH National Human Genome Research Institute. DNA Sequencing Costs: data. Available from: https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data [Last accessed on 27 Sep 2023].
35. Incerti D, Xu XM, Chou JW, Gonzaludo N, Belmont JW, Schroeder BE. Cost-effectiveness of genome sequencing for diagnosing patients with undiagnosed rare genetic diseases. *Genet Med* 2022;24:109-18. DOI PubMed
36. Implications of whole genome sequencing for newborn screening-a public dialogue. Available from: https://files.genomicsengland.co.uk/documents/public-dialogue-wgs-for-nbs-final-report.pdf [Last accessed on 27 Sep 2023].